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The Relationship between Nitrogen Dioxide Concentrations in the Atmosphere Measured by the Sodium Arsenite Method and the Chemiluminescence Method

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Abstract: The relationship between the nitrogen dioxide concentration measured by the Sodium Arsenite method and the Chemiluminescence method, aimed at developing a device for measuring nitrogen dioxide in the atmosphere by means of the Sodium Arsenite method and studying the correlation between the concentration of nitrogen dioxide in the atmosphere which was measured by Sodium Arsenite method and the Chemiluminescense method done by the Pollution Control Department, Thailand. In this research sampling collection of nitrogen dioxide concentration in various conditions by the Sodium Arsenite method was compared with that by the Chemiluminescence method done by the pollution control Department at the Air Quality Measurement Station at the Din Dang district, Bangkok. It was done by one-hour average value totaling of thirty data. The research result found that the sampling collection with the flowing rate of 160 ml/min and the absorption reagent with mixture of 0.2 M sodium hydroxide and 0.015 M sodium arsenite had the highest relationship to the measurement result done by the Pollution Control Department at a statistical significance level of 0.05 with the value of correlation coefficient at 0.658 which had a high relation.

Keywords: Air pollution sampling, Chemiluminescense, Nitrogen dioxide, Sodium arsenite.

Introduction

The air pollution problem in Bangkok Metropolitan has been an important concern and has become more violent especially concerning problems of fine particulate matter (PM – 10 and PM - 2.5), carbon monoxide (CO), nitrogen dioxide (NO₂), etc. Nitrogen Dioxide was the same type as the gas in a high reaction called "oxides of nitrogen or NOx". This gas occurs when there is fuel combustion with high temperature. Nitrogen dioxide is one of the most common air pollutants in ambient air (Hanninen *et al.*, 2004; Lai *et al.*, 2006).

Oxide of Nitrogen's important source was from combustion with a high temperature as in Table 1, both from natural existence and manmade occurrences. The Oxides of Nitrogen by combustion was categorized into three types (De Nevers, 2000). • Oxide of the Nitrogen caused from the combustion at the temperature of higher than 1,300 degrees Celsius is called "thermal NO_x".

• Oxide of the Nitrogen caused from nitrogenous substances in fuel combustion is called "fuel NO_x "

• Oxide from Nitrogen that occurs from the reaction between molecules of nitrogen and the free oxidants of HCN, NH, and N in the flame is called "prompt NO_x " which occurs at a low temperature.

The major outdoor source of NO_2 concentrations are mobile and stationary combustion sources (Lewne *et al.*, 2004; Kampa & Castanas, 2008). Oxides of nitrogen were emitted from vehicles and factories of many kinds such as power plants, cement plants, electronic plants, etc.

Nitrogen dioxide is mainly formed from the oxidation of Nitric oxide (NO) emitted from fuel combustion. Long-term exposure to NO_2 can

lower a person's resistance to respiratory infections and aggravate existing chronic respiretory diseases. (Air Science Group, 2012). Nitrogen dioxide is a toxic gas and is thus a regulated in Thailand. Nitrogen dioxide with high concentration will directly jeopardize the lungs; for example, it irritates the lungs, irritates alveoli and results in decreasing the function of the body's immune system which causes pneumonia and lung cancer, causing constricted emphysema, and finally respiratory system infections such as the Asian flu. If the nitrogen dioxide reaches 300-500 ppm, it causes life threating conditions, coma, or even death, because the brain lacks oxygen. This is because Nitric Oxide merges with hemoglobin in the same way as Carbon Monoxide which causes a decrease of oxygen in the blood. However, in the atmosphere, there is less of a nitric oxide concentration; it is less than 1.22 mg/ M^3 (1 ppm). Thus, it is not dangerous to a human being's health. However if Nitric Oxide is at the concentration level of 0.7 - 20 ppm in 10 min, a human being will not be able to breathe and if it is at the level of 0.11-0.22 ppm, a human being will be able to smell it. Nitrogen dioxide affects a human being's health more than nitric oxide does at the same concentration level.

Table 1. The concentration of nitric oxide occur-ring at different temperatures.

Temperature (°C)	Concentration (ppm)
20	0.001
427	0.30
527	2.00
1,538	3,700.00
2,200	25,000.00

Source: Suadee, 2003.

The present measurement method used in various countries and also Thailand is the standard measurement and that is equivalent to the measurement method used in the U.S. EPA, which defines the nitrogen dioxide measurement method by the Chemiluminescence method as a standard method, and with the Sodium arsenite method as well as TGS-ANSA as equivalent methods. (Choo-in, 2013)

The samplings and the measurement by the Sodium Arsenite method is used mainly by passing air sampling through sodium hydroxide (NaOH) with sodium arsenite (NaAsO₂) becoming a nitrite ion (NO₂). The nitrite ion quantity occurring can be measured by the colorimetric method or spectrophotometric analysis at 540 nanometers after reaction with phosphoric acid, sulfanilamide and 1 - napthyl ethylene diamine dichydrochloride or NED respectively (Choo-in, 2013). A purple-red azo dye is produced. Ultra Violet–Visible spectrophotometer was used for analysis. The spectrophotometer is calibrated against different concentrations of standard solutions of sodium nitrite (NaNO₂) at a wavelength of 540 nm. (Lodhi, 2006).

The principle of the Chemiluminescence method is the measurement method or the spectrum analysis occurring from the chemical reaction for the measurement of all oxides of nitrogen (NO_x), nitric oxide concentration first, and finding the balance from the nitrogen dioxide concentration. In the first step, the nitric oxide has to be measured before by the principle that nitric oxide will react with the ozone and produce nitrogen dioxide and oxygen. A part of the occurring nitrogen dioxide will be in the stimulating status and back down immediately together with the release of spectrum energy (photon) as in the equation (1) - (3). This spectrum energy produced is the direct property to nitric oxide quantity which can be measured. The oxide measurement of all the nitrogen can be done by transforming oxide of other nitrogen to be nitric oxide and measuring all nitric oxide quantity which will equal to the value of all the oxide of all nitrogen. Then the electronic circuit in the measurement device will calculate the concentration value of nitrogen dioxide that was found firstly to be deducted from the oxide concentration value of all nitrogen after being transformed as a form of nitric oxide from the various oxide forms (Pollution Instrumentation, Inc, 1995).

 $NO + O_3 - > NO_2 + O_2$ (1)

- $NO + O_3 > NO_2^* + O_2 \dots (2)$
- NO_2^* ----> $NO_2 + h\upsilon$ (3)

Because the measurement device which uses the Chemiluminescence method is very expensive, this research aimed at the study of the relationship between the nitrogen dioxide concentration measured by the Sodium Arsenite method and by the Chemiluminescence method of the Pollution Control Department of Thailand in order to apply it in the measurement of nitrogen dioxide in the future.

Materials and Methods/ Methodology

I. Instrument

Instrument for the sampling collecting (Fig. 1) comprises:

- Impinge
- Pump for air sucking
- Air flowing rate calibrator of dry type
- A thermometer and barometer
- Spectrophotometer: at 540 nanometer





II. Chemical substances

• Absorbing reagent was 0.05M sodium hydroxide, (NaOH) and 0.004M sodium arsenite (NaAsO₂). Preparation was done by dissolving 0.5 g. of sodium arsenite and 2 g. of sodium hydroxide in deionize water and diluting to 250 m. in the volume metric flask. It should be kept in a light brown bottle.

• Sulfanilamide solution can be prepared by dissolving 20 g. of sulfanilamide in 700 ml. of deionize water. Slowly add 50 ml. of phosphoric acid (85% H₃PO₄) and dilute it with the deionize water to 1,000 ml. in the volumetric flask. This reagent will remain unchanged for one month if chilled.

• Hydrogen Peroxide (H_2O_2) solution can be prepared by dissolving 0.2 ml. of 30 % Hydrogen Peroxide solution and diluting it with deionize water to 250 ml. in the volume metric flask. This solution will remain unchanged for a month if chilled in the dark. • N-(1- napthyl) ethylene diaminedichydrochloride (NED) is prepared by dissolving 0.5 g. of N-(-1napthyl) ethylene diaminedichydrochloride in deionize water and diluting it to 1,000 ml. in the volumetric flask. This solution will remain unchanged for a month if chilled in the dark.

• Nitrogen dioxide standard solution. The preparation used sodium nitrite (NaNO₂) at the purity level of at least 97% NaNO₂. Sodium Nitrite Stock Solution can be prepared as follows: precisely weigh NaNO₂ at the amount of 0.1 mg. and the weight of the substance as follows in equation (4)

$$G = \frac{1.5 \times 50}{A} \dots (4)$$

Definitions:

G is the weight of NaNO₂ (g) 1.5 is the gravimetric conversion factor A is NaNO₂ in the chemical substance (%)

Sodium nitrite was diluted with deionize water to 1,000 ml. in the volume metric flask. This solution will contain the concentration of 500 mg of NO₂ per ml.

• Sodium nitrite working standard (10 mg/ml. of NO₂ (can be prepared by pipette 5 ml. of stock solution NO₂ and dilute it with deionize water to the 1,000 ml. in the volume metric flask. Freshly prepare it when it is to be used.

III. Research Methodology

• Sampling implementing by studying of the flowing rate of air samplings (140, 150, and 160 ml/min) and the appropriate concentration of the absorption solution by the Sodium Arsenite method with 9 experimenting sets are in Table 2.

• Collection of nitrogen dioxide according to the components in each experimenting set as an average value of each hour with the amount of 30 samples. The study was done at the Air Quality Measurement Station (Din Dang Station) of the Pollution Control Department (PCD), Bangkok. This station used the Chemiluminescence method.

• The study of the correlation between nitrogen dioxide concentration value measured by the Sodium Arsenite method and the Chemi-luminescence method by using the principle of correlation statistics was at the confidence level of 95%.

Table 2. The experimental set to find the appropriate factors in the measurement of Nitrogen dioxide by the Sodium Arsenite method.

set	Air flow rate (mL/min)	Absorption substance	Concentration of absorption substance (M)
1	140	Sodium hydroxide	0.05
1	140	Sodium arsenite	0.004
2	140	Sodium hydroxide	0.1
2	140	Sodium arsenite	0.008
2	140	Sodium hydroxide	0.2
5	140	Sodium arsenite	0.015
4	150	Sodium hydroxide	0.05
4	150	Sodium arsenite	0.004
5	150	Sodium hydroxide	0.1
5	150	Sodium arsenite	0.008
6	150	Sodium hydroxide	0.2
0	150	Sodium arsenite	0.015
7	160	Sodium hydroxide	0.05
	100	Sodium arsenite	0.004
0	160	Sodium hydroxide	0.1
0	100	Sodium arsenite	0.008
0	160	Sodium hydroxide	0.2
9	100	Sodium arsenite	0.015

Research Result

The result of the correlation of appropriate experimental set 0.2 M sodium hydroxide mixed with 0.015 M sodium arsenite at concentration level of to test the correlation between concentration value measured by the Sodium Arsenite method and the Cheiluminescence method by using the correlation statistics. It was found that they had correlation and the highest correlation coefficient value was 0.658 and it was at the statistical significance level at 0.01 as in Table 3 and Figure 2.

Table 3. The result on the relationship between nitrogen dioxide concentration measured by the Sodium Arsenite method in each experimental set.

set	N	Sig value	r
1	30	0.140	0.267
2	30	0.005*	0.504
3	30	0.945	0.013
4	30	0.417	0.154
5	30	0.034	0.389
6	30	0.069	0.337
7	30	0.226	0.337
8	30	0.002*	0.538
9	30	0.000*	0.658

Remarks: * means there was a relationship at statistical significance at the level of 0.01.



Figure 2. Correlation of the nitrogen dioxide concentration measured by the Sodium Arsenite method (in experimental set 9) and the Chemi-luminescence method.

The regression result between nitrogen dioxide concentrations measured by the Sodium Arsenite method in each experimental set is shown in Table 4.

Table 4. The regression result between nitrogen dioxide concentrations measured by the Sodium Arsenite method in each experimental set.

	Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		В	Std. Error	Beta		
1	Constant	21.962	5.460		4.023	.000
	set 9	.012	.003	.658	4.626	.000

a Dependent Variable: CHEMI2

The regression equation can be written as in equation (4):

$$(NO_{2(chem)}) = [0.012 \text{ x} (NO_{2(So)})] + 21.962 \dots (4)$$

When

 $NO_{2(So)}$ is the concentration of nitrogen dioxide measured by the Sodium Arsenite method (ppb).

 $NO_{2(chem)}$ is concentration level of nitrogen dioxide measured by the Chemiluminescence method (ppb).

0.012, 21.962 are constant values.

Conclusions

The research result found that the sampling collection with the Sodium Arsenite method which used the air flowing rate at 160 mL/min and used the absorption substance that was a mixture of 0.2 M sodium hydroxide at the concentration level and 0.015 M sodium arsenite at the concentration level of for one hour will showed the highest relation value and concentration value measured by the Pollution

Control Department with an r value was 0.658 which showed a rather high relationship.

The regression study result was utilized in adjusting the concentration value of nitrogen dioxide measured by the Sodium Arsenite method to be approximate to the measurement result gotten from the Chemiluminescence method of Pollution Control Department. The regression equation can be written as equation (5) as follows.

 $NO_{2(chem)}$) = [0.012 x ($NO_{2(So)}$)] + 21.962(5)

When

 $NO_{2(So)}$ is the concentration level of nitrogen dioxide measured by the Sodium Arsenite method in (ppb).

 $NO_{2(chem)}$ is the concentration level of nitrogen dioxide measured by the Chemiluminescence (ppb).

0.012, 21.962 are constant values.

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Study of the Electric Energy Consumption of the Science Center Building

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Abstract: This research aimed to study the status of energy consumption in terms of electric energy consumption and specific energy consumption (SEC) of the Science Center Building, Suan Sunandha Rajabhat University, Bangkok, Thailand. It was found the electric energy consumption in 2012 was 77,818 kWh. The specific energy consumption was 27.91 MJ/m². It needed to have an energy conservation measure for the electric system for short-term measures. For the long-term, the electric system installation was regularly improved and maintained.

Keywords: Electric energy consumption, specific energy consumption.

Introduction

Electric energy involves to the daily life of human beings. The increasing of world population effected on high energy usage. In 2012, the commerce accounted for high electricity consumption was as follows: department stores, hotels, apartment, retail trade and real estate services, respectively. This involved an increase of the GDP about by 6.5 and final energy consumption of 5.2 kTOE (Department of Alternative Energy Development and Efficiency, 2010, 2012). Conventional energy is mostly used for the electric energy generation which has an effect on decreasing the energy resources. Furthermore, decreasing energy resources will lead to the crisis of an energy shortage that directly affects rising energy costs and fluctuations in energy prices. Therefore, all countries realize the problem and try to solve the problem by reducing energy consumption or choosing energy equipment.

The situation of the energy in Thailand in 2012 showed that the energy consumption was higher than in 2011 by about 3.9% (Department of Alternative Energy Development and Efficiency, 2010, 2012). The trend of energy usage in 2013, based on the economic growth in the country, was approximately 4.5 to 5.5% with global crude oil prices about 108 to 113 dollars per barrel. The electric consumption in 2013 increased with the commercial energy margin by about 5.4% when compared with 2012. For the

electric energy consumption in buildings, an energy conservation law for buildings with a transformer size from 1,000 kW or more was enacted. The energy management for buildings was reported to the Ministry of Energy. The report showed the energy usage of buildings and guidelines for saving energy. Furthermore, the Prime Minister's Cabinet promoted a policy of energy conservation by reducing the energy use in government buildings. The Prime Minister's Cabinet had agreed to closely monitor the situation in global energy prices and found ways to mitigate the economic impact. In a cabinet meeting on March 16, 2012, the government had to reduce energy consumption by at least 10 % in order to reduce oil imports (Energy Policy and Planning Office, 2013). Pochkao (2005) researched energy savings in the education buildings of Vongchavalitkul University in Nakhon Ratchasima province, Thailand (Pochkao, 2005). This research studied and evaluated the energy consumption in the terms of the lighting systems, air conditioner systems and other systems. The energy usage could be divided into three categories such as lighting (14%), air conditioner systems (75%) and the other systems (11%).

This research was to study the energy consumption and specific energy consumption (SEC) of the Science Center Building, Suan Sunandha Rajabhat University, Bangkok, Thailand. After the study, guidelines for energy management were determined.

Methods

This research was conducted by collecting the data of electric energy consumption of science center building, Suan Sunandha Rajabhat University, Bangkok, Thailand in 2012. The data were then analyzed to determine energy consumption. The details of the research were shown as follows:

Collection of Data

The data of electric energy and information of the area of the Science Center Building were collected.

The data was gathered by surveying, observing and studying the behavior of the electric appliances, staff, students, and general users. Types of electric equipment of the science center building were also surveyed. The data of electrical equipment were collected. - The equipment and the behavior of user, staff, students, the electric devices such as electric lamps, air conditioning systems, and other systems in everyday use were explored.

- Information (air conditioner systems and a label campaign to switch off lights after use) from surveying the area around the building was implemented. The temperature of the air conditioner was not to be set below 25 °C.

Data Analysis

- The data of electric energy consumption and the information of area layout were analyzed.

The specific energy consumption (SEC) could be calculated from the determination of the ratio of the amount of electricity (kWh) and total area.
The specific energy consumption was calculated using the following equation:

SEC = [electric energy consumption (kWh) x 3.6]

total area
$$(m^2)$$

Analysis of the theoretical approaches for saving the energy was done for the determination of what measures could be implemented for energy savings in the building in the short and long term.

Results and Discussion

A. Area of the Science Center Building

The west-side of the Science Center Building was near a parking and exposed to natural light. It directly exposed to the sun in the afternoon which resulted in the increasing of temperature in the building. The south-side of the Science Center Building could take the advantages of natural light and wind. The north-side of the building connected to the Social Sciences Building. The east-side of the Science Center Building was shaded by tall buildings which blocked the wind and natural light in the morning.

The area of the science center building is divided into seven floors. There are various rooms, such as classrooms, laboratories, conference rooms, computer rooms, the Office of the Dean, a library room, a staff room, a storage room, parking and restrooms. The total area of the university is 128,001.14 square meters (m²). The area of the Science Center Building is 10,038 m².

The area of the Science Center Building is divided to two parts such as the air conditioned area of $6,216 \text{ m}^2$ and the non-air conditioned area of $3,822 \text{ m}^2$. The electric system for air conditioners, lighting and other systems were 1,584.25, 68.16 and 162.14 kW, respectively.



Figure 1. The area of Science Center Building compared with the university.

Those of areas the Science Center Building were divided into two parts: such as the air conditioned areas of 62% and non-air conditioned areas of 38% as shown in Figure 2.



Figure 2. The area of the air conditioned and non-air conditioned areas of the Science Center Building.



Figure 3. The electric power load in the Faculty of Science and Technology.

Figure 3 shows the proportion of the electric load in the science center building. It was divided into three systems, such as air conditioners, lighting and other systems, about 87%, 4% and 9%, respectively. The "other" systems consisted of official equipment (computers, printers and projectors).

Table 1. The proportion of electrical devices on each floor.

Floor	Total area (m ²)	Air conditioner system (kWh)	Light system (kWh)	Total (kWh)
1		1,861.14	91,04	1,952.18
2		2,025.22	87,64	2,112.87
3		2,072.10	92,45	2,164.55
4	10,038	2,653.41	98,08	2,751.49
5		4,062.15	176,77	4,238.92
A1- A2*		NA	20,45	20,45
B1- B2*		NA	16,99	16,99
Total	10,038	12,674.02	583,42	13,257.44

Note: NA is not applicable.

* A1-A2, B1-B2 is the parking lot.

Table 1 shows the proportion of electronic devices on each floor of the Science Center

Building. The 5^{th} floor had the greatest electronic load (kWh), because it was used for the offices of all lecturers and classrooms. All of the area on the 5^{th} floor was the air conditioned area. On the other floors, it showed the electronic loads (kWh) were smaller than the 5^{th} floor, because they had the laboratory rooms which were non-air conditioned area.

B. The Energy Consumption of Science Center Building

The electric energy consumption of the Science Center Building on each floor was considered. The energy usage was different for each floor. Thus, the energy requirement for each activity was not equal and depended on the activity. Two systems were considered, such as air conditioner systems of 12674.02 kWh, and lighting systems of 583.42 kWh.

The energy consumption of the Science Center Building is shown in Table 2.

Table 2. The energy consumption and energy cost of the Science Center Building compared with the university.

	Science Center Building		Suan Sunandha Rajabhat University		
Month	Electric energy	Price	Electric energy	Price	
	(kWh)	(THB)	(kWh)	(THB)	
Jan	7,54	28,459.22	859,54	3,255,774.74	
Feb	6,061	22,848.74	926,68	3,653,987.80	
Mar	6,497	24,455.91	726,669	3,796,505.90	
Apr	6,497	24,455.91	313,389	1,102,000.32	
May	6,27	23,663.33	861,294	3,226,924.38	
Jun	6,479	25,052.71	1,421,927	5,658,128.61	
Jul	6,479	25,887.47	1,082,032	4,293,891.67	
Aug	6,479	25,887.47	985,722	3,923,280.72	
Sep	6,27	26,260.31	812,089	4,007,111.78	
Oct	6,497	30,422.35	352,049	1,581,012.29	
Nov	6,27	29,434.78	1,277,451	5,755,237.84	
Dec	6,479	30,422.35	618,087	2,727,029.46	
Total	77,818	317,251.00	10,236,929	42,980,885.51	

Table 2 shows the relationship between electric energy consumption and electric cost in 2012 of the Science Center Building and all of the buildings in the university.

The highest power consumption occurred in January, because there were events, including a New Year festival and building maintenance. June and November were the beginning of the semesters. April and May were the summer semester. The amount of energy use in other months remained similar because of regular teaching schedules. In each month, the energy use was not consistent with the amount of electricity cost.

C. Specific Energy Consumption of Science Center Building

The specific energy consumption (SEC) involved the energy usage and the production unit. The production unit of the building used a living space. Monitoring and controlling of the SEC were the way to manage building energy conservation.



Figure 4. The specific energy consumption of Science and Technology Building in 2012.

Figure 4 shows the specific energy consumption of the Science Center Building in 2012. The SEC of January was equal to 2.70 MJ/m^2 . It shows that the energy consumption was the highest because of maintenance of the building. Other months had an average SEC of 2.29 MJ/m^2 . The SEC of the building was related to the events, weather at the university, and opening-closing during the semesters. This result corresponded to the energy consumption of the Science and Technology Building of Sripatum University (Srimode, 2011).

Conclusions

The SEC of the science center building was equal to 27.91 MJ/m². The analysis of energy use could provide a method for saving money and energy conservation measures. Conservation measures were divided into two phases: the short and long term. It required using energy conservation measures for air conditioner systems, lighting and elevators in the short-term. In the long-term, the electrical equipment and electric systems installation were regularly improved and maintained.

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Endophytic Xylariaceae from Thai Plants: A Research Review

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Abstract: Endophytic fungi are present in all plant species investigated, and members of the Xylariaceae are especially common in tropical plants. There is major interest in endophytic fungi, because of their excellent track record concerning the production of novel and often bioactive compounds including the anti-cancer drugs taxol and taxane. Studies in Thailand on endophytes over the past 20 years confirm the high presence of the Xylariaceae in many different Thai plants. Species of *Xylaria* stand out as the most frequent Xylariaceae isolated; although, *Daldinia eschscholtzii* has also been commonly isolated. DNA technology and chemical profiling have been shown to be invaluable in the identification of xylariaceous isolates which previously could not be identified to species level or even assigned to genera in many cases. Reference is made to those isolates which produce novel compounds or those exhibiting bioactive properties.

Keywords: endophytic fungi, xylariaceae, Thailand, novel metabolites.

Introduction

Endophytic fungi have been described as those organisms that live inside the plant tissue for at least part of their life cycle without causing any disease symptoms in the host (Petrini, 1991). There is currently major interest in endophytes mainly following the discovery of the anticancer drugs taxol and taxane in the endophyte Taxomyces andreanae Strobel, A. Stierle, D. Stierle & W. M. Hess (Stierle et al., 1993) and subsequent references to bioactive compounds isolated from endophytes (Strobel et al., 2004; Tenguria et al., 2011). It is noteworthy that the Xylariaceae are well known as a source of novel metabolites (Whalley & Edwards, 1995; Stadler & Hellwig, 2005) but also exhibit a remarkable presence as endophytes being regular and often dominant members of the endophytic communities of tropical plants (Petrini et al., 1995). It is therefore not surprising that the Xylariaceae have been the target for many researchers. In Thailand, there have been a number of important studies with numerous publications on both novelty of

the chemicals produced and their bioactive properties. Early studies on xylariaceous endophytes encountered difficulties in identification, certainly to species level, since they fail to produce stable diagnostic features and most of the anamorphic endophytic isolates failed to develop their teleomorph in culture. In most cases, the cultures and anamorphs are insufficiently distinctive to enable confident identification but pioneering work by Petrini and colleagues (e.g. Petrini, 2013; Petrini & Petrini, 1985; Petrini et al., 1995) has resulted in the development of keys and publication of suitable data to allow identification to be made at least to generic level for temperate isolates. The situation regarding tropical endophytic Xylariaceae is much more complex as a result of their abundance, almost universal presence and their impressive diversity (Rodrigues & Samuels, 1990; Rodrigues, 1994; Whalley, 1996). It is doubtful whether differentiation of species on the basis of cultural and anamorphic features alone will ever be possible since differences between individual species are often insufficient to allow

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for absolute identifications to be made (Petrini et al., 1995). Attempts were made to use a combination of morphological and biochemical data and these were considered to be promising (Brunner & Petrini, 1992; Rodrigues et al, 1993). During this period, investigations into secondary metabolite profiles from endophytic isolates might be 'matched' with those obtained from cultures derived from teleomorphic material thus enabling identity to be established (Whalley & Edwards, 1995; Whalley, 1996). In a few cases, identification could be made on the basis of metabolite profiles compared with those from known teleomorph forms. Since then, Stadler and many co-workers have developed his а sophisticated database of secondary metabolites occurring in an impressive range of xylariaceous taxa using HPLC-DAD/MS profiling, HPLC-DAD/MS de-replication, GC-MS profiling and NMR spectroscopy (Stadler et al., 2001a, 2001b, 2008, 2010). A recent monograph of the genus Daldinia Ces. & De Not. combining traditional taxonomic characteristics with chemical and molecular data provides powerful evidence for this approach and the great future potential for the identification of these previously unidentifiable xylariaceous endophytes (Stadler et al., 2014).

In this mini review, we refer to important aspects of the lifestyle of endophytic fungi concentrating on our studies in Thailand over the past 20 years. Previously unpublished data is included and is discussed in the light of other studies on endophytic fungi.

Endophytic Xylariaceae of Thailand

In early studies of endophytes of Tectona grandis L (teak), attempts were made to produce the teleomorphs of a range of endophytic Xylariaceae to enable identifications to be made. It was found that inoculation of pre-sterilized twigs with pure cultures isolated from mature stromata on decaying plant material collected from the sites where endophytic isolates had been obtained or from cultures of selected xylariaceous endophytes which when placed in the forest to develop could often produce mature stromata (Mekkamol et al., 1997; Mekkamol, 1998). Daldinia eschscholtzii (Ehrenb.) Rehm took 12-14 weeks. but species of Xvlaria took over 24 weeks or longer to maturity. Xylaria grammica (Mont.) Mont., X. cubensis (Mont.) Fr. and D. eschscholtzii were subsequently recognized from mature stromata developing in twigs inoculated

with endophytic isolates. Interestingly D. eschscholtzii was found to be the dominant endophyte in teak leaves sampled early in the rainy season with Xylaria later becoming more frequent (Mekkamol et al., 1997; Mekkamol, 1998). It is suggested that this was a result of the higher inoculum potential of the Daldinia ascospores following the development of mature stromata early in the rainy season and its ability to produce large numbers of spores. Xylaria species were found to take longer to develop to maturity and this might explain their low presence early in the rainy season but their greater frequency later on. Our more recent studies confirm that D. eschscholtzii is a frequent endophyte in Thai plants Chareprasert et al., 2006, 2010, 2012). In their studies on endophytes of wild banana (Musa acuminata) at Doi Suthep Pui National Park (Photita et al., 2001) and from Amomum siamense Xylariaceae were also common endophytic fungi (Bussaban et al., 2001). A review of endophytic fungi in Thailand gave insight into their diversity and potential for future investigations (Lumyong et al., 2004) Endophytes from Garcinia species also exhibited high xylariaceous representation (Phongpaichit et al., 2006). A major study of endophytic Xylariaceae in Thailand was then undertaken by Okane et al. (2008) in which 405 strains of Xylariaceae (273 endophytic and 132 saprobic strains) were studied to examine the diversity and taxonomy of endophytes and to explore the relationships between those endophytes and saprobic Xylariaceae in Thailand recognized by their teleomorphic characteristics. In this study in KhaoYai National Park, it was found that Xylaria species were dominant and in analysis of 28S rDNA D1/D2 sequences 21 species of Xylariaceae were recorded inhabiting tropical plant foliage. Apart from Xylaria, Hypoxylon haematostroma Mont. and D. eschscholtzii were confirmed endophytic isolates. Our own studies from various sites and host plants in Thailand support these findings with Xylaria being by far the dominant xylariaceaous genus in most cases (Mekkamol et al., 1997; Chareprasert et al., 2006, 2010, 2012; Pharamat et al., 2013;Ruchikachorn, 2005).

Whalley (1996) reported Anthostomella Sacc., Biscogniauxia Kuntze, Daldinia Ces., Hypoxylon Bull., Kretzschmaria Fr., Nemania Gray, Rosellinia De Not. and Xylaria Hill ex Schrankto have endophytic representation. Members of these genera have been confirmed as endophytes in Thai plants and selected representatives are shown in Figure 1. Other genera since recorded include Annulohypoxylon Y.-M. Ju, J.D. Rogers & H. M. Hsieh and Muscodor Worapong, Strobel & W. H. Hess. The genus Muscodor albus anam. gen. et sp nov. was erected for an endophytic isolate from Cinnamomum zeylandicum and is included in the Xylariaceae on the basis of its molecular similarities (Worapong et al., 2001). It appears to be widely distributed in tropical rainforests and has been recorded from Thailand (Sopalun et al., 2003). The following new species of Muscodor have since been described from

Chiang Mai Province *M. cinnamomi* Suwannarach, Bussaban, K. D. Hyde & Lumyong, *M. musae* Suwannarach & Lumyong, *M. oryzae* Suwannarach & Lumyong and *M. equiseti* Suwannarach & Lumyong (Suwannarach *et al.*, 2010; 2013). *Muscodor* species are of considerable interest since they produce volatile metabolites with promise for biocontrol of spoilage fungi (Strobel, 2010).



Figure 1. Selected xylariaceous fungi. A. *Daldinia eschscholtzii*, B. *Kretzschmaria* sp., C. *Xylaria grammica*, D. *Xylaria cubensis*, E. *X. grammica* culture on PDA medium, F.X. *cubensis* culture on PDA medium. Bars = 1 cm.

Novel chemicals and bioactivity

Our early studies on secondary metabolites of the Xylariaceae demonstrated a remarkable diversity of chemicals with many proving to be novel structures (Adeboya et al., 1995a, 1995b; Anderson et al., 1982, 1983, 1984a, 1984b, 1985; Edwards & Whalley, 1979; Edwards et al., 1988, 1989, 1991, 2001). It was also shown that the presence or absence of certain metabolites and metabolite profiles were of value in making taxonomic judgement (Whalley & Edwards, 1995). Since this early work, Stadler and co-workers have identified numerous new compounds and in many cases have demonstrated useful bioactive properties (Stadler et al., 2001a, 2001b, 2008, 2010; Bitzer et al., 2008). Their results have proven to be invaluable in both species identification and in taxonomic classification. These publications, together with the discovery of taxol and taxane in an endophyte, have resulted in wide ranging investigations of meta-bolites from the Xylariaceae and xylariaceous endophytes. There are too many publications to list all here, but a few selected papers on bio-active properties by Daferner et al., 1999; Singh et al., 1999; Isaka et al., 2000; Quang et al., 2006 provide a useful base to work from. Sodngam et al. (2014) investigated the chemicals and their activity from Xylaria humosa Lloyd recently found in Thailand and provide key references to bioactive compounds from Xylaria species. Stadler and Hellwig (2005) have reported on a wide range of chemicals identified and their bioactive properties.

Conclusions

The Xylariaceae are common and widely distributed endophytes in those Thai plants which have been investigated. The genus *Xylaria* is dominant in most of the studies undertaken but *D. eschscholtzii* is also a regular inhabitant and in certain situations can be numerically the most important. There are a large number of isolates recognized as xylariaceous without identification to genus and species levels. *Xylaria* endophytes are in particular a good source of metabolites, many novel and a number exhibit bioactive properties.

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Species Diversity of Plankton in Suan Sunandha Rajabhat University, Samut Songkhram Campus

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Abstract: The study of phytoplankton and zooplankton in Suan Sunandha Rajabhat University, Samut Songkram Campus by collecting the samples following the seasons: the cool season (December, 2012), the hot season (March, 2013) and the rainy season (June, 2013). The plankton samples were collected from 5 stations by using 70 micrometers mesh size of plankton net and examined the water quality. The results showed that, in total, there are plankton in 48 genera, 77 species which consist of 36 genera, 58 species of the phytoplankton, and 12 genera, 19 species of the zooplankton. The phytoplankton: Class Bacillariophyceae was the dominant group and the most diverse was the genus *Chaetoceros* (8 species). The zooplankton: Phylum Sarcomastigophora had the most species diversity and most diverse zooplankton was the genus *Ceratium* (5 species). The cool season was the season when the greatest species diversity of the plankton could be found and the water temperature average was 27.79 °C. The pH average was 7.82. The dissolved oxygen average was 6.21 mg/l. The salinity average was 24 ppt. These conditions are the appropriate environment for these living aquatic organisms.

Keywords: Species diversity, Plankton, Samut Songkhram.

Introduction

Plankton are the tiny organisms that are floating in the water resources and they consist of the phytoplankton and the zooplankton. Plankton have diverse morphology that it can be found in every type of water source: fresh water, brackish water and seawater (Ariyadej et al., 2004; Teanpisut & Patarajinda, 2007) Phytoplankton is important producer of the food chain while the zooplankton is the primary consumer which transmits the energy to other aquatic organisms in the food chain and the food web in the aquatic ecosystem. The varieties of environment factors affected plankton community structure (Liu et al., 2010). Both the phytoplankton and the zooplankton are the important food sources for aquatic organisms, so the abundance of plankton can affect organism diversity. Meanwhile, the rapid growth of the plankton or the toxins from some plankton species can both directly and indirectly affect water sources. Some plankton can be harmful to living organisms and also to human beings. The abundance of plankton was

also used to evaluate water resources for being an indicator for the quality of the environment.

Samut Songkhram Province is located in the central region on the west coast of the Gulf of Thailand. This province is divided into 3 districts: Amphawa District, Mueang Samut Songkhram District and Bang Khonthi District. The population's employment consists of agriculture and gardening, such as in the coconut groves, the fruit gardens, fisheries and culturing aquatic animals along the coastal area. Most of Samut Songkhram Province consists of low plains; however the coastal area length is 23 kilometers. There are 3 seasons which are the hot season, the period beginning around February until May; the rainy season, this period starts around May and lasts until October; and the last one is the cool season, beginning around October and lasts to the middle of February.

The purpose of this study was to research the species diversity of the phytoplankton and the zooplankton in the area of Suan Sunandha Rajabhat University, Mueang District, Samut Songkhram Province and use this study as the database of the biological diversity and as a guideline for the sustainable use of biological resources.

Materials and Methods

The species diversity study of the phytoplankton and the zooplankton was conducted at Suan Sunandha Rajabhat University, Mueang District, Samut Songkhram Province at latitude 13° 25' 22.8" N, and longitude 100° 02'14.1" E. This area is close to the coast and influenced by the tide-seawater. Furthermore, there are the mangrove forests in which Avicennia sp. is the dominant species. In the study, water samples were collected in each season from 5 stations (Figure 1), and during 3 periods, being representative of each season. In the cool season, collecting the samples was in December 2012; March 2013 was the period for collecting the samples in the hot season; and the rainy season, the samples were collected in June 2013 by towing 70 micrometer mesh size of plankton net at the depth level of 30 centimeters from the surface of the water. The water samples were fixed with 4% buffered formalin for preservation of the samples. The plankton was identified by using a compound microscope and the samples were photographed. The study of the phytoplankton was made using the reference of Wongrat (1999). The study of the zooplankton was made using the reference of Wongrat (1998) and World Register of Marine Species (WoRMS). In addition, there was an examination of environment factors: the water temperature, pH, salinity, and the dissolved oxygen (DO), which were measured by use of a thermometer, pH meter, salinity refractometer and DO meter, respectively.



Figure 1. The stations for sample collection.

Results and Discussion

The study of species diversity of the phytoplankton and the zooplankton in Suan Sunandha Rajabhat University, Bang Kaeo Sub-district, Mueang District, Samut Songkhram Province consisted of research in the cool, the hot and the rainy seasons; the samples were collected one time each season from 5 stations. The result of the study revealed a total of 48 genera, and 77 species of the plankton were found which could be divided into phytoplankton in 3 divisions, 36 genera, and 58 species; consisting of Division Cyanophyta: 4 genera 4 species of Class Cyanophyceae, Divison Chlorophyta: 3 genera, and 3 species of Class Chlorophyceae: Division Chromophyta: 27 genera, and 51 species of Class Bacillariophyceae. However, the most abundant

phytoplankton found was Class Bacillariophyceae: 51 species or 66.23% of the total plankton found and the second most abundant was Class Cyanophyceae: 4 species (5.19%), and the least abundant was Class Chlorophyceae: 3 species (3.89%). The phytoplankton which were the most abundant were *Chaetoceros* (8 species) and the second-most abundant was *Nitzschia* (5 species). When comparing results with Teanpisut & Patararajinda (2007), they reported that *Chaetoceros* were the most common genera in Koh Chang, Trat Province, which is similar to this studied.

Most of the phytoplankton could be found in the cool season, when 38 species were found; the second-most was in the rainy season, when 34 species were found, and the season that the fewest phytoplankton were found was in the hot season, when 24 species were found. That result is different than Arkronrat et al. (2012), who reported in Prachuap Khiri Khan Bay, that the most phytoplankton could be found in the rainy season. For the station rank in the collection of the most phytoplankton species was the first station, with 41 species; the second one was the fifth station with 40 species, the third one was the fourth station with 34 species; the fourth one is the second station with 23 species; and the station with the fewest species of phytoplankton was the third station with 17 species.

The study found zooplankton consisting of a total 4 phyla, 12 genera and 19 species which

consist of 6 genera and 11 species of Phylum Sarcomastigophora, 4 genera and 5 species of Phylum Ciliophora, 1 genus and 2 species of Phylum Rotifera, 1 genus and 1 species of Phylum Arthropoda. In addition, the study found 3 groups of the zooplankton which are cyclopoid copepods, copepod nauplii and cirripede nauplius. The most diverse zooplankton found were 11 species or 14.28 % of the Phylum Mastigophora; the second-most diverse was 5 species (6.49%) of Phylum Ciliophora and the third-most diverse was Phylum Rotifera 2 species (2.59%) and the least diverse was Phylum Arthropoda 1 species (1.29%) (Figure 2). The genus of the zooplankton which was the most diverse was *Ceratium* (5 species) and the second-most diverse was Favella and Brachionus (2 species). The season in which the greatest diversity of plankton was found was in the cool season, in which 15 species were found; the second greatest diversity was found in the hot season, in which 8 species were found; and the least diversity of zooplankton found was in the rainy season: 5 species. The station rank for finding of zooplankton is as follows: the most diversity was at the fourth station with 12 species; the second most diversity was the first station with 11 species; the fifth station had 9 species; the second station had 8 species; and the station with the least diversity of zooplankton was the third station with 4 species (Figure 3).



Figure 2. Percentage of plankton species in Suan Sunandha Rajabhat University, Samut Songkram Campus.



Figure 3. Number of plankton species in each station.

In comparison with the types of the plankton in the area of Mueang District, Samut Songkhram Province, the ratio of the phytoplankton is greater than the zooplankton ratio. The phytoplankton totaled 58 species or equal to 75.32% of the complete plankton found. The zooplankton totaled 19 species or equal to 24.68% of the totally plankton found (Figure 4). The comparison of the number of the species of plankton, the zooplankton had a smaller number of the species than the phytoplankton. Collection of the samples should be made with different sizes of the plankton nets to get a greater variety of plankton. Moreover, this study used a plankton net with a mesh size of 70 micrometers which may have had an effect to the number of plankton species collected.



Figure 4. The ratio of the phytoplankton and the zooplankton.

The results of the plankton species collection at Suan Sunandha Rajabhat University, Samut Songkhram Campus found that it had a similar result as the previous reporter, in which diatoms in Division Chromophyta (Class Bacillariophyceae) were the dominant group of the phytoplankton in lotic sites (Al-Saadi et al., 2000; Wu et al., 2011). This study also found all seasons were similar to the study of Arkronrat et al. (2012). The plankton found in this period of the study was calanoid copepods, cyclopoid copepods and copepod nauplii which were all the varieties of plankton groups found most. Furthermore, copepod are the plankton which indicated the abundance of biodiversity in the water sources and are the important food for aquatic organisms (Drillet et al., 2011).

The study of the environmental characteristics found that the water temperature average in the period of the study was 27.79 degrees Celsius (ranged from 25.0-29.4 °C). The highest water temperature average was 29.04 °C in the hot season. The water temperature average in the cool season was 27.82 °C, and the rainy season recorded the lowest water temperature average was 26.50 °C. The salinity average was 24 ppt (ranged from 19-28 ppt). The highest salinity average was 25 ppt in the hot season. The salinity average in the cool season was 24 ppt and the rainy season which the lowest salinity average was equally 23 ppt. The pH average was 7.82 (ranged from 6.60-9.05). The highest pH average was 8.87 in the rainy season. The pH average in the cool season was 7.42, and the lowest was in hot season when the pH average was equal to 7.16. The dissolved oxygen average was 6.21 milligram per liter (ranged from 5.53-6.92 mg/l). These environmental factors values was in standard level of the National Environment Committee Announcement (Ministry of Science and Technology, 1994) The cool season had the highest dissolved oxygen average equal to 6.91 mg/l. The dissolved oxygen average in the hot season was 6.00 mg/L. In the rainy season, there was the lowest dissolved oxygen average which was equal to 5.71 mg/l.

Season	Water temperature (°C)	Salinity (ppt)	рН	Dissolved oxygen (mg/L)
Cool season	27.82	24	7.42	6.91
Hot season	29.04	25	7.16	6.00
Rainy season	26.50	23	8.87	5.71
Average	27.79	24	7.82	6.21

Table 1. Average of environmental characteristics in different seasons.

Conclusions

The species composition of the phytoplankton and the zooplankton in the area of Suan Sunandha Rajabhat University, Samut Songkram Campus were studied by collecting samples of the plankton following the cool, the hot, and the rainy season. The samples were collected from 5 stations that found plankton in 48 genera, and 77 species, which consisted of the phytoplankton in 36 genera, and 58 species; the zooplankton consisted of 12 genera, and 19 species. Class Bacillariophyceae had the most species of phytoplankton and genus *Chaetoceros* (8 species) had the greatest variety species. Phylum Sarcomastigophora was the zooplankton which had the most species diversity, and genus *Ceratium* (5 species) had the greatest variety of species. The cool season was the season when the most species of plankton could be found, and the water temperature average was equal to 27.79 degrees Celsius. The salinity average was 24 ppt. The pH average was equal to 7.82. The dissolved oxygen average was 6.21 milligram per liter and the results indicated that these conditions were an appropriate environment for the living aquatic organisms.

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GIS Application in Urban Traffic Air Pollution Exposure Study: A Research Review

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Abstract: This paper reports a preliminary study of the evaluation and forecast of transport-related air pollution dispersion in urban areas with the help of Geographic Information System (GIS) platform and a simulative system with graphical interface. The urban population growth, economic development, energy consumption, growing transportation demand and living standards play major role in pollution exposure in atmosphere. A lot of research has already been done to investigate the functional relationship between air quality and air pollution from transport. This study is an effort to develop a more flexible framework of model to find the exposure of the air pollution in the atmosphere. This review article describes the development of framework of different GIS inputs that help to find the exposure of vehicular pollution in megacity.

Keywords: Dispersion, Pollution Exposure, GIS Application, Urban Air Quality, Vehicular Emission.

Introduction

In current scenario the air quality remains one of the major environmental issues in modern society (Tiwary & Colls, 2010). Quality of air affects the entire human race as well as plant and animal populations on the earth. The population growth, economic development, growing transportation demand as well as living standards contribute major role to polluting the surrounding area and atmosphere (Onursal & Gautam, 1997). Urban areas produce complex gases and particulates whose characteristics depend upon a wide range of factors such as: population density, energy consumption, industrial processes, and modes of transportation and usage, which affects public health, damages agriculture, weather, and climate (Pant & Roy, 2012). At present, urban outdoor air pollution causes an estimated 1.3 million deaths per year worldwide, according to the World Health Organization (WHO, 2012). The annual growth rate of the vehicle population in India is around 10% during the previous year (Nagpure et al., 2013). The GIS helps to monitor the various temporal scales and thus can reduce the cost and time of field surveys, which would play a crucial role in conservation of environment. The objective of this paper was to find out the exposure of vehicular pollution to society with the application of GIS.

Emission of toxic pollutants from motor vehicles

Motor vehicles produce more air pollutants than any other single human activity. Motor vehicle emissions from highways might be viewed as a mobile line source with an emission rate per unit length of highway. In a regional scale model, the consolidated emission from an entire urban area might be viewed as an area source with an emissions rate per unit area. In city centers and congested streets, traffic is responsible for 80% to 90% of these pollutants and this situation is particularly severe in cities of developing countries (Whitelegg & Haq, 2003; Batterman et al., 2014). Emissions from motor vehicles with spark ignition engines are from the exhaust, engine crankcase, and fuel system. Carbon dioxide (CO_2) and water vapor (H_2O) , the main products of combustion, are emitted in vehicle exhaust (Onursal & Gautam, 1997). The major pollutants emitted from gasoline-fueled vehicles are carbon monoxide (CO), Hydrocarbons (HCs), Oxides of Nitrogen (NO_x) and lead (Pb). In most industrialized countries, people who live in urban areas tend to be more affected by allergic respiratory diseases than those who live in rural areas. Road traffic is the main contributor to air pollution in most urban areas and there is evidence that living in the vicinity of high traffic roads is associated with impaired respiratory health. Transportation of freight and passengers, especially in Delhi, is primarily dependent on road traffic. As a consequence of the high traffic density in Delhi, the average speed within the city is relatively low (Gurjar *et al.*, 2008). Large quantities of CO₂, CO, HC, NO_x and particulate matter (PM) are emitted regularly from motor vehicles. The mobile air toxins such as benzene, formaldehyde, acetaldehyde, and lead, along with secondary aerosols can cause adverse human health impacts (Amato *et al.*, 2010).

Factors affecting vehicular emission

Real world vehicular emissions are highly variable. Several factors account for the variability in emissions in different vehicles and the amount of environmental damage they can cause. However, due to relatively higher average temperatures, poor fuel quality, poor vehicle maintenance culture, and a high proportion of old vehicles, the level of emissions from mobile sources are usually high. Figure 1 shows the various factors that are major cause for vehicular emissions in the transport sector.



Figure 1. Factors influencing the environmental damage due to release of air pollutants.

Worldwide scenario of air pollution

Worldwide road transport vehicles like cars, trucks, and buses play a significant role in air pollution. In the European Union road vehicles are the largest sources of CO, NO_x and nonmethane hydrocarbons. The recent trends indicate that road vehicles are responsible for 74% of NO_x and 94% of black smoke emissions in London (USEPA, 2010). According to the United States Environmental Protection Agency (US-EPA, 2010), diesel vehicles account for 32% and 87% of total emissions for these two pollutants respectively. Likewise, due to rapid vehicle increases, vehicle emissions have now become a major environmental concern in China (Huo *et al.*, 2011). In Indonesia, 70% of air pollution is

due to transportation activity and 30% comes from industrial activity (Sutanto *et al.*, 2005). The WHO reported that the two thirds of death occur-red due to the outdoor air pollution in Asia. In 2010, particulate air pollution in Asia led to over 2.1 million premature deaths and 52 million years of healthy life lost. The outdoor air contributes to 1.2 million deaths in East Asia and 712,000 deaths in South Asia.

The World Health Organization (WHO) 2014 reported that the urban air quality database covers 1600 cities across 91 countries which are facing air pollution health risks. In April 2014, the WHO reported that outdoor air pollution was responsible for the deaths of 3.7 million people under the age of 60 in 2012. The numbers of automobiles increased at a rapid rate in megacities during last decades in India. Delhi is regarded as one of the most polluted city in the world. Annual growth rates of motor vehicles are continuously increasing due to rapid motorizetion. The most disturbing factor is the rising density of the vehicles in urban area. The rising density of vehicles is responsible for the emissions of different vehicular pollutants like CO, NO_X , SO_X , PM_{10} and $PM_{2.5}$.



Figure 2. Annual mean of PM_{2.5}µg/m³ in various countries (Source: WHO Report, 2014).



Figure 3. Annual mean of $PM_{10} \mu g/m^3$ in various countries (Source: WHO Report, 2014).

Figure 2 represents annual mean concentration of particulate matter of size 2.5μ m. Various countries that exceeded the National Ambient Air Quality Standards (NAAQS) were Pakistan, Qatar, Afghanistan, Bangladesh, Iran, Mongolia, United Arab, India, Bahrain and Nepal. The lowest concentrations were observed in Lebanon. The people are also suffering due to the risk of serious health problems in these countries.

 PM_{10} have higher annual mean concentrations *i.e.* more than $100\mu g/m^3$. The human population is more exposed to PM_{10} in the respective countries. The figure 3 depicts the annual mean concentrations of PM_{10} in different countries. Some countries that exceeded the NAAQS are Pakistan> Afghanistan> Bahrain> Senegal>Qatar >Bangladesh> United Arab Emirates> Mongolia >Egypt >India >Jordan> Iran >Nepal. The lowest concentration of particulate pollutants was found in Lebanon.

Trends of air pollution in India

The outdoor air pollution has been listed as one of the top ten killers in the world, with 65 percent of the air pollution deaths occurring in Asia and close to quarter of this in India. The Central Pollution Control Board (CPCB) conducted a study in six major cities i.e. Delhi, Kanpur, Bangaluru, Pune, Chennai and Mumbai and found more than a 30% contribution of these cities in ambient air quality. The major cities like Delhi, Mumbai, Chennai, Kolkata, Bengaluru, Hyderabad and Ahmedabad, as well as secondary cities with populations of more than 2 million like Pune, Surat, Indore, Bhopal, Nagpur, Jaipur, Varanasi, Nagpur, Agra, Guwahati, Patna, Kanpur, Panaji, Trivandrum and Cochin are growing both geographically as well as in population, putting pressure on local infrastructure and life style. The increase in traffic along with inadequate infrastructure facilities is responsible for the emissions of air pollution and greenhouse gas emission (GHGs) emissions. Vehicular pollution is a major contributor towards the increase of air pollution in urban cities with respect to domestic and industrial sources, according to the Central Pollution Control Board (CPCB 2012). The following chart shows the contribution of different sectors towards the ambient air quality (Figure 4).



Figure 4. Contribution of various sectors to ambient air quality in urban city (Source: CPCB, 2012).

In addition to this, the annual mean of PM_{10} and $PM_{2.5}$ in different Indian cities are presented in figure 5 and 6. In figure 5, the highest concentration of $PM_{2.5}$ was found in Delhi; whereas, the highest concentration of PM_{10} was observed in Gwalior (Figure 6). Warangal city was found in much better condition in comparison to other Indian cities due to far less concentrations of $PM_{2.5}$ and PM_{10} (Figure 5 and Figure 6).



Figure 5. Annual mean of $PM_{2.5} \mu g/m^3$ in various Indian cities.



Figure 6. Annual mean of $PM_{10} \mu g/m^3$ in various Indian cities (Source: WHO Report, 2014).

The World Health Organization (WHO) estimates that air pollution contributes to the deaths of approximately 800,000 people annually (WHO, 2012). Different studies of megacities on different spatial scales used various modeling tools to understand their local-to-regional-toglobal impacts and implications (Guttikunda et al., 2001; Gurjar & Lelieveld, 2005). Outdoor air pollution causes approximately 1.3 million deaths every year worldwide (WHO, 2011). Around 40 million people within the 115 largest European cities are still exposed to air quality exceeding the European Union (EU) limit values for at least one pollutant (WHO, 2012). In megacities, people travel everyday using different types of vehicles. The daily increase in the number of people in megacities results in increased traffic volume. Every person uses vehicles according to their life style. The different types of vehicles in urban cities are buses, autos, light commercial vehicles, heavy commercial vehicles, Car/Jeep and 2-wheelers are playing a major role in the air pollution in mega cities. The buses travel more in a day, they emit more pollution and two-wheelers emit less pollution. According to a local survey, 30% of Delhi's population is suffering from respiratory disorders due to air pollution. The respiratory disorders increased 12 times as compared to all other states of India. Due to that, Delhi is characterized as the "asthma capital" of India (Guttikunda et al., 2012; Das et al., 2004). Increases in deaths due to air pollution have made air pollution the fifth leading cause of death in India, with 620,000 premature deaths. This is up from 100,000 in 2000, a six-fold increase (WHO, 2014). Below, figure 5 and figure 6 show the annual mean of particulate matter in various cities in India.

GIS application in air quality mapping:

A geographic information system (GIS) is a computer-based tool for mapping and analyzing geographic phenomenon that exist and occur on earth. Yan et al. (2009), Rytkonen et al. (2004) and Hammond et al. (2011) utilized GIS to study the community level pollutant concentrations and associated health risks. They explored Toxic Assessment, Air Quality System, and National Emission Inventory for identifying risks of exposure to air pollutants at the community level. GIS basically integrates baseline data and unique visualization of geographic analysis on the maps. It is very useful to acquire the pollutant spatial distribution, variations and characteristics information because of its flexible spatial data management and effective spatial data analysis methods. It is an innovative and important component of many projects for public health and epidemiology studies. GIS may also involve more sophisticated spatial analysis of disease occurrence and contributing environmental factors. Scoggins et al. (2004) used model concentrations were converted from point base grid coverage to polygon grid coverage using GIS. Polygon grid coverage concentrations were then converted to census area unit concentration. It creates very informative graphical presentation and is easy to understand. Sahzabi et al. (2011) also conducted a study to predict CO₂ dispersion and optimization of the dispersion model. GIS is used for visualizing model results and better analyses of CO₂ concentration status at a particular geographical area. Morality rates were calculated using the number of people exposed to pollution at census area unit level. It plays a vital role in the planning of air quality management in megacities. In present scenario the GIS techniques are used for exposure air quality assessment (Nuckols et al., 2004). It simulates traffic, emission, and dispersion patterns of the pollution. The GIS and modeling characteristics are presently used for air quality assessment. The various sub layers are represented different in figure 8, including topographical, land use data, transport network infrastructure, socioeconomic, demographic data, traffic data, different air pollutants, and environment impact parameters. The analysis of transport planning and transport systems management can also be done through GIS technology. The GIS maps show a combination of data layers as presented in Figure 8. The GIS database is systematic on the basis of geographical base line data, which consists of inputs of attributes in the GIS software. The road corridor network map of the study area is digitized with GIS software. These linked maps are integrated with a number of various topographic morphological attributes of the study area. In particular, the whole territorial database, which has been set up within GIS, is designed according to the details of inputs. The use of GIS as a database integrator for a transport study area and schematic diagram represents a set of individual databases for the study area, which comprise a mixture of spatial, numerical, and perhaps textual data. The GIS is a very dynamic technology capable of integrating large quantities of geographic spatial data. The GIS database is provided by road networks corridor data, traffic demand characteristics, driving cycles and fleet composition. GIS integrating models are aimed at reproducing traffic behavior, emission and dispersion scenarios (Elliott & Wartenberg, 1997).



Figure 8. Layout of superposition of data layers in GIS.

Materials and Methods

The principle of Geographical Information System (GIS) is basically superimposed of all spatial data and attributes of the study area. Figure 9 shows the different sub spatial data of thematic map data, transportation network data and population data. Each road link contains attribute data, which is required by the air pollution simulation model. Figure 8 also shows a combination of various input parameters, which help to develop the various models. All these models are integrated step by step with GIS and help the spatial mapping of the air pollution. The information is imported to the GIS software action to spatial datasets. The result shows the overlay to display, the intersection of sub systems as a geographical map. The base line data was collected from the different air pollution monitoring stations. The other data like meteorological parameters, different types of pollutants and their concentrations are necessary for the GIS integration for air pollution assessment.



Figure 9. Air pollution exposure modeling with integration of GIS.

The base line monitoring data from pollution monitoring station include pollution concentration of various pollutants. Pollutant concentration is measured in grid zone of the study area like 2KM X 2 KM grid plot. It could be monitored more accurately and ideally using gridbased methods such as Discovery Net Project (Richards *et al.*, 2006). Spatial interpolation could be implemented in many types GIS software, such as Geomatica10, ArcGIS10.2. Exploratory Spatial Data Analysis (ESPA) is carried out according to the characteristics of the data before interpolating in order to choose the suitable interpolation methods.

Figure 10 show the mapping of the $PM_{2.5}$ and PM_{10} in the Delhi NCR. The distribution of vehicle exhaust and the brick kilns. The dark brown color shows the maximum concentration of the particulate matter. In the study, South and Central Delhi was found to be most affected zone due to particulate matter pollution.



Figure 10. Annual average percentage of $PM_{2.5}$ and PM_{10} form the vehicle exhaust and brick kilns emission for year 2010 in Delhi, India (Source: Guttikunda *et al.*, 2013).

Figure 10 show the mapping of the $PM_{2.5}$ and PM_{10} in the Delhi NCR. The distribution of vehicle exhaust and the brick kilns. The dark brown color showed the maximum concentration of the particulate matter. In the study, south and central Delhi is found most affected zone due to particulate matter pollution.

Emission and dispersion models

Air quality models are used as mathematical, numerical techniques to simulate the physical, chemical processes that affect air pollutants in the environment. The different inputs like meteorological parameters, source information and emission rates of pollutants are used to characterize the primary pollutants emitted directly into the ambient air. All these models are important for the assessment and management of air quality of environment. It is very useful to identify source contributions to air quality problems and to assist in the design of effective strategies to reduce harmful air pollutants. The air quality models can also be used to predict future pollutant concentrations from multiple sources (US-EPA 2012).

The various emissions of pollutants and dispersion models simulate the pollutants. This traffic related pollutants are major source of air pollution in the urban traffic corridor. The emission estimation of various pollutants is found out with the help of emission model. The emissions of the entire vehicle fleet are calculated on the basis of emission coefficients, traffic volume, and road segments (Equation 1).

$$E_i = L \times C_i \tag{1}$$

Where E denotes the emissions of pollutant i, L denotes the vehicle kilometers traveled, and C denotes the emission coefficient of pollutant i. Dispersion models simulate the dispersion of pollutants after its emission. Line source Gaussian plume dispersion model is originally used to predict the impacts of carbon monoxide near different transport corridor. The Gaussian solution for linear sources which is based on is based the principle of overlaying various component, which assume the concentrations of emitted substances at the receiving equal to the sum of dispersions of all infinite points sources which is together in line source.

$$C(x, y, z) = \frac{Q}{2\pi u \sigma_y \sigma_z} exp\left(-\frac{y^2}{2\sigma^2 y}\right) \left[exp\left(-\frac{(z+H)^2}{2\sigma^2 z}\right) + exp\left(-\frac{(z-H)^2}{2\sigma^2 z}\right)\right]$$
(2)

Source of emission at ground level,

$$C(x,0,0) = \frac{Q}{2\pi u \sigma_y \sigma_z}$$
(3)

Where

C(x,y,z) is the concentration unit (gram/meter³) at some point in space with coordinates x,y,z Q= the emission rate of the pollution source (in gram/second)

u= the average wind speed in (meter/second)

 σ_y = the standard deviation of the plume in the y direction (meter)

 σ_y =the standard deviation of the plume in the z direction (meter)

Emission and dispersion models can be carried out using integration of GIS and environmental models. Emission models are used for the various transport corridor which gives traffic volume, gridding zone of the study area with suitable cell-size raster, finding the cells that intersect with road segments. The output raster shows the emission rate from the source area. The 2 KM X 2 KM divided as girding in the study area with suitable cell raster that calculating each cells value second equation using second equation. The obtained raster indicates the spatial distribution of pollution concentration.

Land use regression

Land use regression (LUR) combines monitoring of air pollution at a small number of locations and development of stochastic models using predictor variables usually obtained through GIS software. The regression model is then applied to a large number of locations in the study area. The input data include traffic characteristics of different land use area, physical geography. (David *et al.*, 1997; Gerard *et al.*, 2008).The climate variables such as wind speed direction and wind speed has significant effect on pollution dispersion distance. The climate variables are neglected when using LUR; it will bring an error to value of the predictor variables that are computed from the circular zones around each monitoring site. Gulliver et al. (2011, 2005) evaluated GIS-based dispersion model Space Time Exposure Modeling System-Air pollution (STEM-Air) to simulate traffic related air pollution. Behera et al. (2011); Mion et al. (2002) developed an emission inventory for all sources responsible for PM₁₀ pollution in Kanpur, India, with the then used ISCST3 dispersion model to simulate PM₁₀ concentrations to identify pollution hotspots in the city and to evaluate the contribution of each source in PM₁₀ pollution level. Elbir et al. (2004) developed a decision support system for air quality management in big Turkish cities. The developed GIS system was based on the CALPUFF dispersion model and related databases to calculate emissions from various sources. Pollutant concentrations were predicted using the CALPUFF dispersion model and results were compared with observed air quality data from actual monitoring stations. After model calibration, GIS was used for air quality mapping and scenario analysis.

As shown in figure 9, the GIS is integrated with different sub models, which aimed for stimulating each sub models that process involved in the entire air pollution phenomenon. These are traffic emission estimations, dispersion models all linked together. The survey input of all geographical information which is important for the study area. Road traffic is simulated through a deterministic model designed for solving the equilibrium auto assignment problem with capacity constraints. The emission model has been developed on the basis of input data and it is able to calculate emission levels of some typical traffic-related pollutants such as carbon monoxide (CO), hydrocarbons (HC), and nitrogen oxides (NO_x). Emission factors refer to a vehicle grouping based on the knowledge of vehicle model year, fuel type, gross weight, and power characteristics. All these input parameters are very useful for the assessments of air quality as well as for the developed model.

Exposure analysis application

Exposure assessment describes the damages to the human health, plant ecosystems and sensitive areas like hospitals, schools, residential area. Vehicular pollution exposure modeling on human is very spatially complex and temporally dynamic process. The simulation is necessary for simplification and plays a key role in implement exposure modeling (Borrego *et al.*, 2006; Gulliver *et al.*, 2005). GIS provides an effective way to implement exposure modeling. Exposures are estimated for each location, at each time interval, by cross-reference to the pollution concentration raster for that time period as shown in equation 4.

$$E_{ijk} = C_{ij} \times W_k \qquad (4)$$

Where E denotes the exposure of an individual during time i, at location j, in exposure environment k. C denotes pollutant concentration during time i at location j and W denotes the weighting factor for exposure environment k, relative to ambient. Modeling exposure uses raster-based calculation. Mapping exposure is carried out using map algebra operation. Exposure profiles are carried out extracting grid values on exposure map along the moving path using combination of several tools in Geomatica10.

Results and Discussion

This paper helps to examine the assessment of vehicular emissions with the help of advanced modern technology based study like GIS. Spatial distribution of urban air pollution concentration is very useful from a technology based assessment. The spatial patterns of pollution resulting from policy or other interventions and provide improved estimates of exposure for epidemicological studies. Delhi has recorded the highest increase per capita road length decrease and more importantly the number of vehicles per km of road has tremendously increased. This indicates the number of vehicles registering has increased fivefold in Delhi. The rapid growth of personalized transport in Indian cities gives rise to serious issue due to increment of air pollutant

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problems. In the current scenario, Delhi is facing the highest vehicular emission load. In recent times, Delhi NCR is rapidly modernizing and commercializing. Figure 2 shows the air pollution mostly due to vehicular emissions which contributes 72% and the industrial sector contributes 20% in the megacities of India. This shows that the road transportation has a major role in air pollution. The considerable magnitude of air pollution increases the number of people suffering from respiratory and cardiovascular diseases. That is the major issue which results in premature deaths in India. There is a need to control population growth and vehicular air pollution in the megacities. Special efforts should be made for educating the general mass and local leaders about the adverse effects of a large population and vehicular pollution through specially designed information, education and communication activities. Upgrading the quality of Indian fuel, enforcing higher emission standards and regulating traffic can reduce the pollution caused by the explosion in the number of automobiles in megacities. Those who use personal vehicles should be encouraged to use the public transport system. State transport service should set up better vehicular standards, and more research and development should be encouraged in vehicle technology. Air pollution should not be a responsibility of government alone but mass and local leaders should be encouraged to make dedicated efforts to eradicate the air pollution problems. The policy aimed at overall development should certainly include efforts to control population, private vehicles, and air pollution for better health of the present and future generations.

Conclusions

In present scenario the developing as well as developed megacities are polluting the ambient air and disturbing the natural environment system. It also increases public health issues. It is necessary to learn comprehensive processes and ways to study urban pollution. This paper represents the air quality modeling and evaluating the impact of vehicular pollution with advanced technology. In this study, the integrated modeling approach involving GIS has been used for pollution mapping of vehicular pollutants in megacities. It monitors various temporal scales, which will reduce the cost and time of field surveys. It is most suitable for the local administration in order to forecast alert pollutant levels in air quality area. It also gives the integrated

existing monitoring network measurements and estimates the sensitivity of pollution levels to such variables as traffic flow distribution and atmospheric conditions. The interpretation of satellite data is more complex as compared to insitu observations. Satellite data sets should be of good quality, easily accessible and reliable for the assessment of air pollution. It is very useful in the model-based approach and the predictions for the epidemiology remain to be seen. The desired future development of GIS requires a switch of emphasis from data and information to knowledge of the atmospheric conditions. The results help in the developments of methods for the study of ambient air pollution and air pollution assessment. GIS has been proved a powerful tool to implement the methods involved in the framework and makes the methods more efficient and flexible. Urban air pollution research will be further developed with increasing concerns of urban planners, developers, health officials and citizens living in the city. The GIS framework is an extension of new methods for the environmental conservation of future.

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Development of Animation Teaching Media on the Topic of *The Property of The Father*

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Abstract: The objective of this research was to develop animation teaching media on the topic of *The Property of the Father* to teach for students to understand the concept of sufficiency. The animation was developed to evaluate the performance of a sample of 50 grade 4-5 students at Wat Don Sali School. The results were as the achievement of the students who were taught with the animation was .05 significantly and students had a high level of satisfaction with the animation.

Keywords: Animation, teaching media, sufficiency.

Introduction

Humans have different ways of learning. Some can assimilate in a better way the knowledge received visually, auditory or through a certain sense. Educational systems generally provide a unique and standardized teaching material such as books to all learners which tend to benefit to those whose learning style and background knowledge fits well with the teaching material. If the teaching style employed closely matches the student preferred style of acquiring knowledge, learning becomes easier and more natural, results improve and learning time is reduced (Shannon, 2009). On the other hand, if for example a student is more visual than verbal and everything is written on the blackboard without auditory resources, student will experience difficulties in attaining the pedagogical goals in the requested time (Adisor, 2008.). In few words, traditional teaching material and strategies generally tend to benefit some students more than others.

Society today is bringing the benefits of technology, graphics, animation, and application development of such instruction, film and media production, advertising which is very interesting and easy to understand. One major tool set teachers/instructors can use is animation, which presents content in a way that helps students understand content (Patcharin & Suwich, 2012). Animation is a powerful instructional device to help explain and reinforce concepts of content which promote imagination and entrepreneurial skills to come up with fun and enjoyment. There is a great amount of research related to learning studies, including behaviorist research, cognitive research, experiential learning, including behaviorist research, cognitive research, experiential learning, humanistic research and social learning. Martyn Stewart (2011) has highlighted the major contributors, and has argued that learning is a complex process that includes both mentally cognitive and emotional affective factors from social and individual perspectives.

The increasing number of students has found that cultural and background differences have influenced their learning processes (Vygotsky, 1962, 1978); these differences have also influenced the design of teaching, which now must take into account the need to help students from very different backgrounds. Cartoons and animation can help students learn concepts correctly and consistently (Sweller, 1994; Akamca et al., 2009). Connor (2009) found that cartoons help students who have difficulty in quickly processing large tracts of written text or dialogues, and increase both learning efficiency and students' interest in learning. This, in turn, has a positive influence on students' achievements (Akamca et al., 2009). Therefore, many teachers have adopted and examined the usage of animation in teaching (Hall, 2005; Becker et al., 2006; Klein & Bauman, 2010; Luccasen & Thomas, 2010). Cingi developed animation to investigate the use of animation in teaching theoretical medical information and especially surgical procedures to teaching a group of 20 medical students. Animation is a very useful tool to teach difficult procedures in medicine especially in teaching complex topics (Cingi, 2013).

The research objective was to develop animation teaching media on the topic of the property of the father to teach students to understand the concept of sufficiency which was a real reflection of the society through the motions. The story talks about on luxuries of everyday society in Thailand. The incentives cause inappropriate behavior to get what they want and get the final result that is a loss of their loved ones because of their insatiability. The hypothesis of the research is satisfaction of the participants to develop animation teaching media on the topic of the property of the father at a high level.

Materials and Methods

A. Defining the Concept of Story

Defining the concept of story sets the featuring of story which is the process of writing the incident from the start and continues to the end. It allows viewers to understand and have a sense that the story is set.

B. Character Design

Character Design is the process of drawing characters on paper to simulate the character of the imagination. The characters are extremely important to make the animation look more attracttive. The character design attempts to make the characters come alive in both appearance and attire (Figure 1).



Figure 1. Character design.

C. Design Background

Design background is considered no less important than the character design. The backdrop

to the atmosphere, as well as the character of a different color and lighting, shall mood and make the audience understand the story to offer (Figure 2).



Figure 2. Design background of school.

D. Defined Outline

Defined outline (Storyboard) is defined as the image into the sequence of the story overview of work to do if anything happens to be solved can be modified to change (Figure 3).



Figure 3. Defined outline.

E. Develop Animation

From defined outline process we will develop animation in part of characters with Paint tool SAI and creates a scene with Adobe Illustrator CS6 software development process animation using Adobe Flash Professional CS6 process of recording the voice of the storyteller. And the voice of the character then cut a piece of work with Adobe Premiere Pro CS6 (Phunchan, 2013) to follow the plot of defined outline and the concept of story (Figure 4-6).



Figure 4. Drawn characters with software.



Figure 5. Draw background with software.



Figure 6. Develop animation.

F. Measurement and Evaluation

The animation was developed to be used as teaching materials to teach for the sample consisted of 50 by selecting a sample of student's grade 4-5 at Wat Don Sali School, Tumbon Don Yai, Aumpur Bangpae, Ratchaburi Province (Figure 7). They were drawn using cluster sampling techniques. After that we measure achievement learning from the pretest and posttest with quiz sufficiency of 10 items by switching the question of the pretest and posttest (Sumitra, 2014). Questions pretest and posttest of finding the ICO from 5 experts that the questions have IOC value greater than 0.5 and we evaluate satisfaction of animation (Terada & Adisai, 2009).



Figure7. Teaching with animation to the sample group.



Figure 8. Achievement learning of a sample uses pretest and post-test.

Results and Discussions

A. Measurement of Achievement Learning

Measurement of Achievement Learning of a sample group of students uses pretest and posttest. Found that higher posttest scores after the first pretest (Figure 8- previous page).

B. Evaluation of Satisfaction

Evaluate satisfaction of animation from a simple group uses the questionnaire with mean and standard deviation. Table 1 shows the list of questions and the level satisfaction levels of students in each part of the questionnaire that came from of a sample group of student's opinion and the overall satisfaction levels of students in each part of the questionnaire of animation is in more satisfaction level.

Question List	Statistics Value and Meaning			
	\overline{X}	S.D.	Quality Level	
1. Contest is clear and easy to understand	3.86	0.60	High	
2. The consistency of content to viewers	3.78	0.67	High	
3. Content can provide insight to the viewer.	3.90	0.61	High	
4. The appropriateness of the use of time of story	3.96	0.75	High	
5. The interest in the animation	4.10	0.70	High	
6. The appropriateness of the use of music and sound effects	4.00	0.50	High	
7. The appropriateness of the use of picture effects	3.94	0.65	High	
Overall	3.93	0.64	High	

Table1. List of questions and the level of animation quality that came from a sample student opinion.

Conclusions

This paper describes the development of animation Teaching Media on the Topic of The Property of The Father to teach self-sufficiency. The sample consisted of fifty 4th-5th year students at Wat Don Sali School, Tambon Don Yai, Amphoe Bangpae, Ratchaburi Province. They were drawn using cluster sampling techniques, then we measured learning and the satisfaction of the sample that an animation developed. The findings revealed the following: 1) the achievement learning of the students who were taught with the animation was significantly .05 higher when comparing the pretest to the post-test; 2) the students had a high level of satisfaction with the animation. The results were consistent with other research that said cartoons and animations can help students learn concepts correctly and consistently (Sweller, 1994; Akamca et al., 2009). Connor (2009) found that cartoons help students who have difficulty in quickly processing large tracts of written text or dialogues, and increase both learning efficiency and students' interest in learning. This, in turn, has a positive influence on students' achievements (Akamca et al., 2009). Therefore, many teachers have adopted and examined the usage of animation teaching (Hall, 2005; Becker et al., 2006; Klein & Bauman, 2010; Luccasen & Thomas, 2010). Cingi developed animation to investigate the use of animation in teaching theoretical medical information and especially surgical procedures to teaching a group of 20 medical students. Animation is a very useful tool to teach difficult procedures in medicine especially in teaching complex topics (Cingi, 2013). Therefore, it can be said the research objectives have been accomplished and the hypotheses of this research have been shown to be valid.

Future Work

For further research, we are leading an animation project for teaching media on the topic of *The Property of The Father* to training and measure learning achievement using pretest and posttest on two group of subject that determine the differences between with and without animation approach.

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Main sections of the text are the introduction, methods and materials/methodology, results and discussion, conclusions, acknowledgements, references, and appendix/appendices. These should be centered. Other text sub-sections should be aligned to the left.

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For a book:

Auffenberg, W. 1988. Gray's Monitor Lizard. University Press of Florida. Gainesville, Florida. 419 pp.

For a chapter within a book:

Sweet, S. S. 2004. Varanus glauerti. In: Varanoid Lizards of the World (eds. Pianka E. R, King D. R.), pp. 366-372. Indiana University Press, Bloomington, Indiana.

Non-English Language References:

Chan-ard T., T. Siangtiangchai, S. Makchai and M. Cota. 2008. Khao Soi Dao Frog: What scientific name should we use? *Ecological Notes* 2(1): 12-13 (in Thai).

For an article found on a website:

Böhme W. (2003) Checklist Of The Living Monitor Lizards Of The World (Family Varanidae). Checklist of CITES Species Compiled by UNEP-WCMC, Convention on International Trade in Endangered Species of Wild Fauna and Flora. http:///www.cites.org/common/cop/12/ESF12i-06A.pdf. (Last accessed 31 January 2006).

An appendix or appendices are added when the author wants to include useful or supporting information pertaining to the article, but it is not necessarily needed in the text.

Tables can be embedded in the text, but must also be submitted separately as a MS Excel or compatible file. Text font should also be in Times New Roman.

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