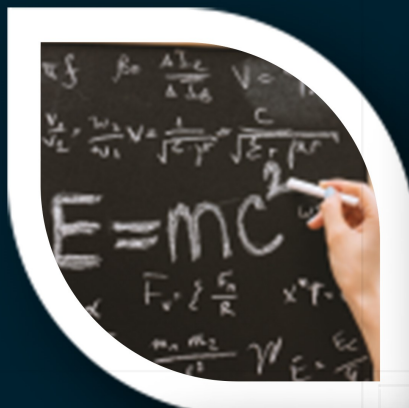


# SSSTJ



Suan Sunandha Science and Technology Journal  
Volume 8, Number 2, July 2021



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# Accommodation Building Designing for Construction Labor Basic Life Quality Promoting

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## Abstract

Since the problem in temporary accommodation for construction labor about space and building for supporting basic living, these are composed as bedding, toilet and showering, cafeteria and sanitation management. These always be necessity facility for life quality of construction labor in Thailand. However, there are the inadequate of designing for space building including facility usage that takes the bad condition of facility usage safety and hygiene. This paper aims to design and show properness of spaces and building in camp site. By questionnaires for 300 sample labors from 3 property construction project in Chonburi. Data were composed as daily activities and need of facilities and analyzed by zone of space. The result showed that proper space and building of camp site were provided as 15 m<sup>2</sup> / 1 labor. These space were separated as the space of accommodation for 0.85 m<sup>2</sup>, sanitation for 1.50 m<sup>2</sup>, emergency for 1.25 m<sup>2</sup>, travelling for 1 m<sup>2</sup>, recreation and retail for 1.25 m<sup>2</sup> and open space for 5.40 m<sup>2</sup>. All content about spaces of camp site were designed as layout of space and building concretely by program sketch up version 8.0. While discussing with various studies and provision about building and facilities for living, it found that there were both agreed and opposed.

**Keywords:** Construction labor, Outside welfare, Temporary accommodation, Lay out of camp site

## 1. Introduction

Due to economic grown extremely at macro level of Thailand by The National Economic and Social Development Plan, the important businesses and activities for country's economic development are constructions these are the beginning point of the businesses considered as their basic factors such as various buildings infrastructure for implementation of businesses and activities, an important part of country's economic development, such as household, factories and commerce buildings for implementing the businesses.

Additionally, the mentioned construction industry does not depend on resources, materials, and technology only. There is also important "construction labor" that takes duty in building construction by skill ability and workforce to build various buildings for usability. These construction labors are not only even human resource taking part for country's economic development, they are also

the consumer taking purchasing power for the economic system of country. Therefore, there are legislation of legal and occupational measurement for safety condition in working, for instance ministerial regulations standard of administration in occupational health for promoting life quality and hygienist of construction labors in making their working task as potential human resources.

Nevertheless, apart from occupational and life quality of construction labor management, there is also facility for basic living human have to do in daily life. These are composed as bedding toilet shower cafeteria construction workers need to use and supported. Those facility for basic living in accommodation need to be designed for proper condition in space ergonomic and hygienic as well as good sanitation management. By mentioned matter, this researcher is interested in basic life quality promoting for construction labors.



## 2. Materials and Methods

Research was implemented by collecting data by questionnaire from 300 labors of 3 property construction projects in Chonburi. These data were composed as daily routine, facilities needs to analyzed and concluded that Ngow (2015) by duration of day and Rojvirasingh (2011) by percentage of needs. Analyzing daily routine activities data in camp site and compiling facilities need data of sample labor to provide size of space usage Sthapitanont (1991) explained for providing space of accommodation facilities for life quality supporting including hygiene in camp site welfare for effective of co-operative space usage. To select 4 items of basic needs facilities to design pattern of building and area by Sketch up program version 8.0.

## 3. Results and Discussions

1. To compile data of facilities need by using a questionnaire for interviewing labor samples of 3 property construction projects jointed research. To explain data of facilities need by Rojvirasingh (2011) studied, the details regarding this are shown in Table 1.

**Table 1.** Shows the result of compiling facilities need of construction labors.

Facilities	Need of labor samples (%)
Accommodation building	100.00
Shower and toilet building	100.00
Kitchen and cafeteria building	75.00
Trash and waste water management	86.67
Emergency usage space	81.00
Route of vehicle traveling area	65.67
Parking area	68.33
Area for exercising	84.00
Area for grocery store welfare	94.33

2. To provide temporary accommodation space, according to the analyzed and concluded data and concerning factors for supporting living convenience and comfort in camp site that provided criteria for space or building per labor by minimum requirement for proper and hygienic living in both short and long term. Regarding this, it showed that 1 labor needed not lower than 15 m<sup>2</sup> of space for temporary building camp site and in space of camp site / 1 labor could be separated as sub-areas like accommodation building area, sanitation usage space, emergency usage space, traveling and recreation and grocery welfare space. Thus, the

space size provided and implemented as temporary camp site for 1 construction labor would be of 9.60 m<sup>2</sup> per 1 person concluded as in Table 2 and Figure 1.

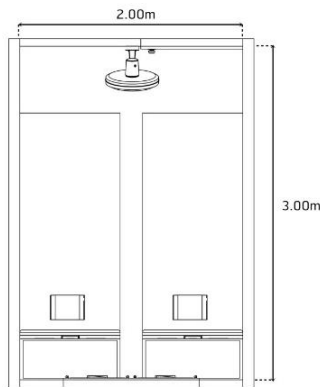
**Table 2.** Conclude size of space used in camp site welfare.

Space for camp site welfare	Space for 1 labor (m <sup>2</sup> )	%
<b>Living welfare space</b>		
Accommodation building	3.00	20.00
Shower and toilet building	1.00	6.67
Kitchen and cafeteria building	1.00	6.67
Total	5.00	33.33
<b>Sanitation usage space</b>		
Building or space for garbage gathering	0.10	0.67
Building or space for trash storage	0.25	1.67
Building or space for waste water treatment	0.50	3.33
Total	0.85	5.67
<b>Emergency usage space</b>		
Assembly point space	0.25	1.67
First aid point space	1.00	6.67
Emergency situation management space	0.25	1.67
Total	1.50	10.00
<b>Travelling space</b>		
Route of vehicle traveling space	0.50	3.33
Parking space	0.50	3.33
Total	1.00	6.67
<b>Recreation and retail welfare space</b>		
Space for exercising	1.00	6.67
Space for grocery store welfare	0.25	1.67
Total	1.25	8.33
All total space usage	9.60	64.00
Open space	5.40	36.00

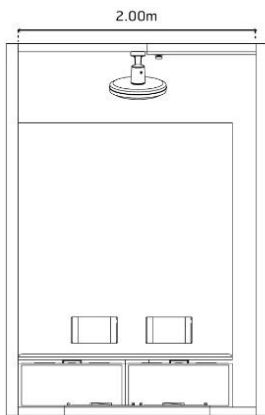
3. To design layout of building and area by data and information in the Table 4 by sketch up program version 8.0 by information as follows.

3.1 Accommodation building by condition that provided not lower than 3 m<sup>2</sup>/ 1 person. This could be designed for room to sleep as 6 m<sup>2</sup> / 2 person (2 X 3 m.) high as 2.50 m. This room space is proper to sleep by a study Rojvirasingh (2011) explained that sleeping behavior includes sleeping in a horizontal position related equipment including bed or mattress for only one person sleeping and a little more space to twist or stretch. The width should be 0.79 m. (Measured from the width of the shoulders at the widest part of the body plus the area for twisting or stretching) The average size of males 0.82 m. and female 0.76 m. Long side are 1.70 m. (Combined with the mean pillow distance from males 1.77 m. and females 1.64 m.). Besides, there must be 1 window in size as 20 % of room space opposite door for ventilation (Figure 3). To store

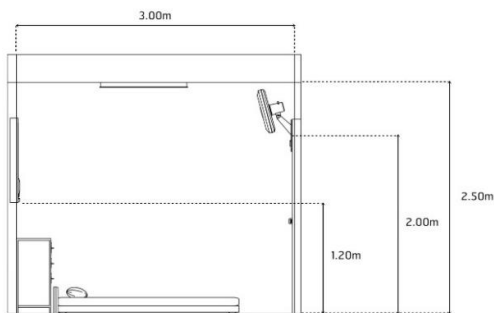
personal stuff such as clothing, toothbrushes, soap, shampoo, etc., mainly using the area above the sleeper's head. It could be a small box or cabinet. The distance between box or cabinet and the wall as about 40-50 centimeters, by Ngow (2015) provided. These could be as figure 1 However labors with families and young children (1-3 years) also can use the space to live and use for other activities in living as well by layout as shown in Figure 1, 2 and 3.



**Figure 1.** Layout in above view shows 2 single labors sleeping room.

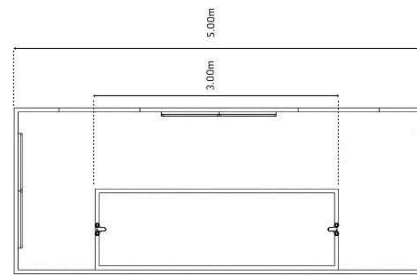


**Figure 2.** Layout in above view shows family labors sleeping room.

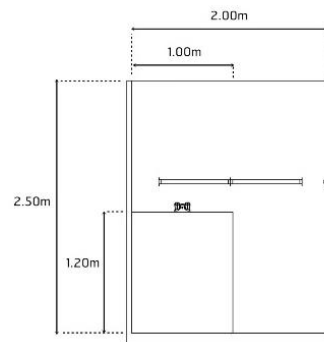


**Figure 3.** Layout in side view shows labors sleeping room.

3.2 Shower and toilet building by condition that provided not lower than 1 m<sup>2</sup>/ 1 person including washing area too. This can be designed as 2 model of shower room. One is single shower room and one is a public bath. For public bath room, there must be a space to construct a room for the labors' bath separated as males and females. This public bath room required a room as 2 x 5 meters, equal to 10 square meters, separate male / female. The height is not less than 2.50 m. Providing tank or reservoir for using of a bowl to scoop the bath in the center or attached to either side of public bath room in size as 1 x 3 m. not higher than 1.20 m. and need size of vent for ventilation and humidity in bath room at least 10% of bath room that must be arranged sufficiently concealed. All mentioned shown as Figure 4 and Figure 5.



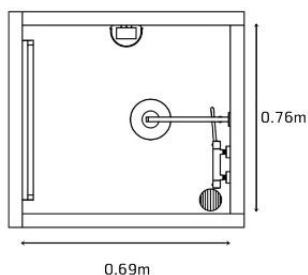
**Figure 4.** Layout in above view shows public bath room and size of reservoir.



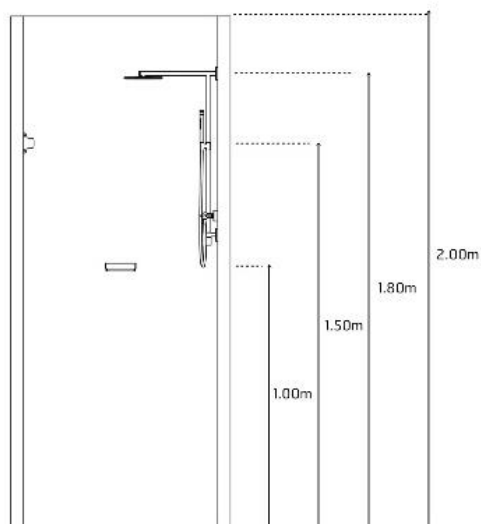
**Figure 5.** Layout in side view shows public bath room.

For shower room may have wide sides ranging from 0.69 m. (measured from the widest shoulder width of the body, combined with the leg placement and the reach of the hand to the soap stand. Average from males 0.72 m. and female 0.66 m.) horizontal distances from 0.76 m. and standing in the shower for reaching a little more average male 0.78 m. and female 0.73 m.), as well as

related equipment such as shower, soap dispenser and handling area. The distance to hold the shower, bend and stand in the shower. Besides, it should be at least 2 meters and must have a vent to ventilate the humidity in shower room at least 10% of the single shower room space that must be arranged sufficiently concealed. All mentioned shown as layout of Figure 6 and Figure 7.



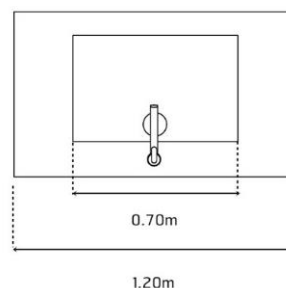
**Figure 6.** Layout in above view shows shower room.



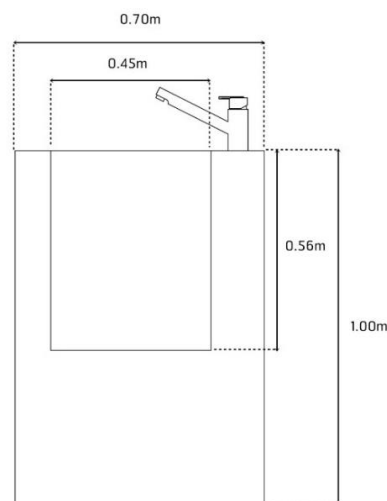
**Figure 7.** Layout in side view shows shower room

Washing area layout by criteria of space not lower than 1 m<sup>2</sup>/ 1 person. Toilets and washing areas are classified as a private area for private activities, but it is an open space for easy entry and exit to engage in activities. Both taking clothes and equipment to wash and taking out to dry after washing finished and also supporting to drain moisture from wet areas as well. In this study provided minimum of 16 m<sup>2</sup> of washing area must be provided for the area in which the sink is established. It was able to set the area with a width of 1.20 m. length 70 cm. the tub size is 70 inches

wide x 45 inches long x 56 cm. high. The finished porcelain which are similar in size as specified by installing in the middle of the washing area. Leave empty spaces on the left and right sides of the tub for resting laundry and washed clothes. Installing the faucet in the center of the inner width, which can be designed as layout in Figure 8 - 9.



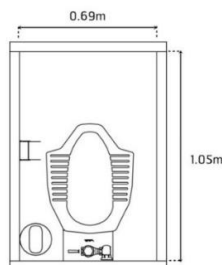
**Figure 8.** Layout in above view shows sink for washing.



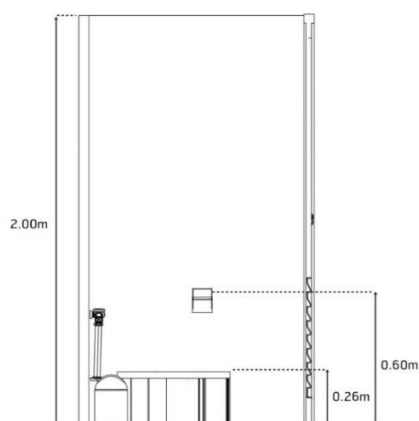
**Figure 9.** Layout in side view shows sink for washing.

Toilet for excretion, building by criteria of space not lower than 1 m<sup>2</sup>/ 1 person, according to Rojvirasingha (2011) explained that toilet usage behavior consists of sitting with related equipment: toilet bowl, bidet sprayer, toilet paper holder. Therefore the width of the toilet should be 0.69 m. (measured from the shoulder distance at it is the widest part of the body, combined with the leg placement and the reach of the hand to the toilet sprayer or toilet paper holder. Average from males 0.72 m. and female 0.66 m.). Long side was 1.05 m. (Measured from the width of the toilet bowl. Combined with a little more leg and reach average

from males 1.11 m. and females 0.99 m.) and the height of the toilets should be at least 2 meters. Details of Toilet for excretion building as layout in Figure 10 - 11.



**Figure 10.** Layout in above view toilet.



**Figure 11.** Layout in side view shows toilet room.

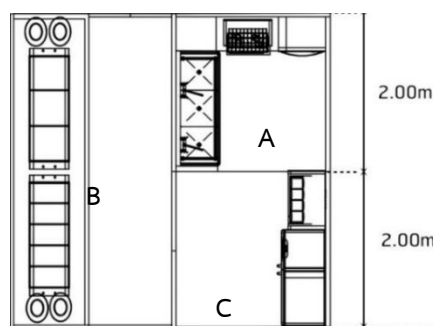
3.3 Kitchen room for cooking by criteria of space not lower than 1 m<sup>2</sup>/ 1 person, since the kitchen is a both place to do activities with cooperation (to store various raw materials that will be used for hygienic consumption) and engaging in conflicting activities (collecting of dirty and contaminated stuff to be washed for further use) In this study, the kitchen building must covered he kitchen and cafeteria. By the reason of hygienic there must divide the area of the kitchen building for cooking into 3 areas as follows

A. Storage area for cooking ingredients set for 5% of the area of the building, kitchen and cafeteria.

B. Cooking area assigned for 10% of building area, kitchen and cafeteria.

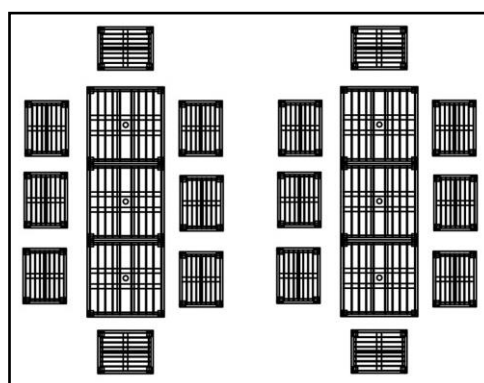
C. Cleaning area kitchen equipment assigned for 5% of area of the building, kitchen and cafeteria.

Details of kitchen room can be designed as layout in Figure 12.



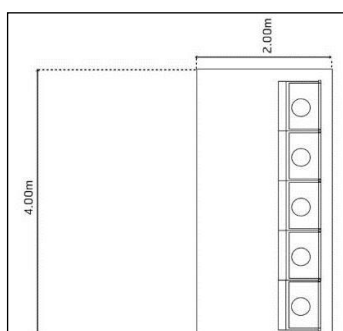
**Figure 12.** Layout in above view shows kitchen room.

Cafeteria building is a building with an area for construction workers' consuming which may be classified as a benefit during breakfast, lunch or evening, depending on the welfare system of the development project. It is necessary to provide a dining area for construction labors, 80% of the area, where equipment and utensils such as tables and chairs may be provided for dining easily and may set aside a portion of the dish and spoon after eating to be tidy before washing in the next cleaning area. Details of cafeteria building as layout of Figure 13.



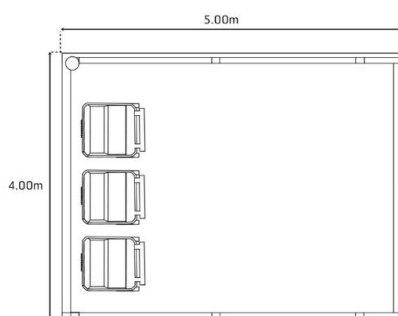
**Figure 13.** Layout in above view shows table and chair position in cafeteria building.

3.4 Building or space for garbage gathering in this study, area for gathering garbage is 2 x 4 m., Equal to 8 m<sup>2</sup>, and may be built into a small tank house to gather garbage from each meal served by the kitchen and dispose of it properly. Sanitation Principles This food waste gathering bin can be used in conjunction with a kitchen building to gather left over raw materials from cooking and may be built as a stall made of wood or other easily demolish materials, not more than 30 cm. in height, to place the garbage bin in Figure 14.



**Figure 14.** Layout in above view shows Building or space for garbage gathering.

3.5 Building or space for trash storage in this study area for trash storage is 4 X 5 meters equal to 20 m<sup>2</sup>, that could be built as a small house for collecting various garbage by classified waste. Sorting bin according to the general community waste sorting principle is sorting recycled material. General and hazardous waste should be properly managed and house should be strictly closed for preventing disease-carrier animals that may be used as habitats as in Figure 15.

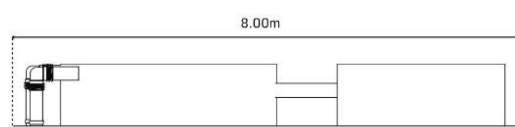


**Figure 15.** Layout in above view shows Building or space for trash storage.

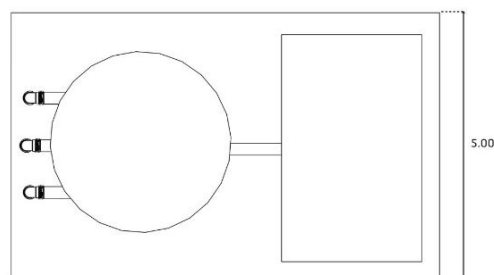
Building or space for waste water treatment In this study an area for wastewater treatment is 8 X 5 m., equal to 40 m<sup>2</sup> being separated as follow

- Wastewater from sewage arising from excreting should build a septic tank system with a suitable capacity for the number of construction workers at least 30% of the area for septic tanks is required for wastewater treatment.
- Wastewater generated from the shower room and kitchen building a well or a water tank should be built for collecting wastewater before draining to public sewerage to enter the wastewater treatment system of the local organization by case of a camp site in an area of waste water treatment

service. For camp site outside service of wastewater treatment system of the local organization, there should be a limited area that does not exceed the criteria. It may be necessary to establish an activated sludge process wastewater treatment system, which can be costly to construct and operate for using in wastewater treatment or used as a stabilized pond wastewater treatment system, constructed wetland wastewater treatment system, oxidation ditch in case of having enough area. For content of waste water treatment is able to be concluded by Figure 16.



**Figure 16.** Layout in above view shows waste water treatment.

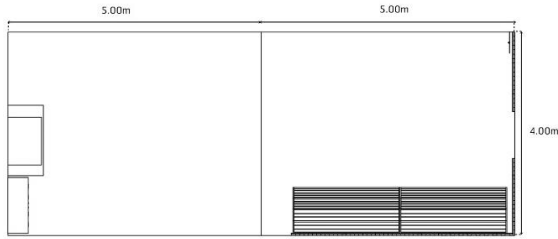


**Figure 17.** Layout in side view shows waste water treatment.

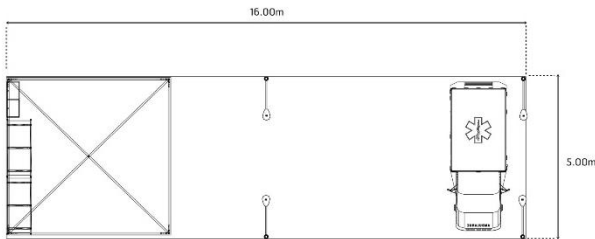
3.6 Size and form of emergency usage space, in this study space to manage emergencies size 120 m<sup>2</sup>, for use of space divided by implementation to support emergency situations as follows

- Assemble area in this case The area for the gathering point is 5 X 4 m. equal to 20 m<sup>2</sup>, that is an open space on the boundaries of an area There may be a label to indicate the area location.
- First aid area, in this case the space for the gathering point is 5 X 16 m. equal to 80 m<sup>2</sup>, which is an open area and may clearly outline the boundaries. And there are signs to clearly indicate the area.
- The area of emergency management, in this case the area of the emergency management is 5 X 4 meters, equal to 20 m<sup>2</sup> being classified as an open area. For details on the Emergency usage space can be designed as layout in Figure 18 - 19.





**Figure 18.** Layout in side view shows assemble point and emergency management.



**Figure 19.** Layout in above view shows first aid point in emergency situation.

3.7 The size and pattern of the traffic route from the assumption, the route has an area of 80 square meters, which can be divided into 2 functional areas as follows

- Vehicle traffic routes, the vehicle traffic route from the doorway is width of 2.50 m<sup>2</sup> and a length of 16 m. that may be made of reinforced concrete or tilde brick paving in the event of long-term use of about 1 year or more in the case of concrete floor or tilde brick laying May also draw lines dividing traffic lanes

- Vehicle parking area from the case of the vehicle parking area is 4 X 5 meters, the vehicle parking area may be set up as an open house and tighten the vehicle parking position.

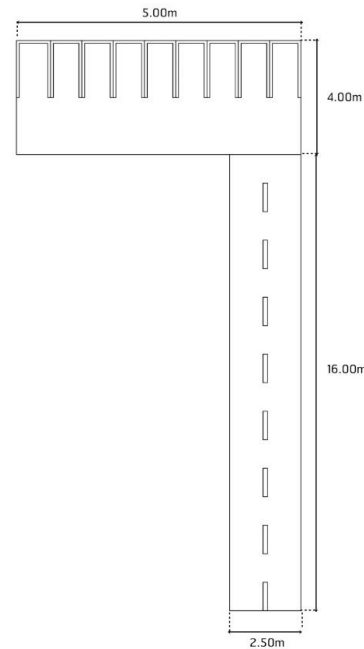
For details on managing traffic routes can be summarized as shown in figure 20

3.8 Size and pattern of recreation and retail welfare space, from the case of recreation and welfare areas, shops and restaurants are 100 m<sup>2</sup>, and the format of recreation areas can be defined as follows:

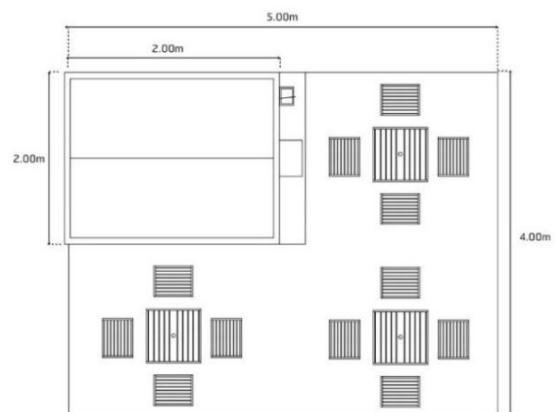
- Space for exercising and recreational activities from this case, the space for exercise and recreational activities is 6.40 X 12.50 m. that may be ground or grassy, not high and not too overgrown to facilitate sports and exercise for construction workers as well.

- Space for grocery store welfare from this case, the space of shop is 4 x 5 meters, equal to 20 square meters, which may be constructed as a temporary shop house with open air for ventilation and product sales. Conveniently By separating the area for a store that can open and close is an area of 4 square meters in the corner of the area other areas may provide a space for tables and chairs for comfortable dining and can be socialized in small groups.

Details of space arrangements for recreation and retail welfare space can be designed as layout in the Figure 20 - 22.

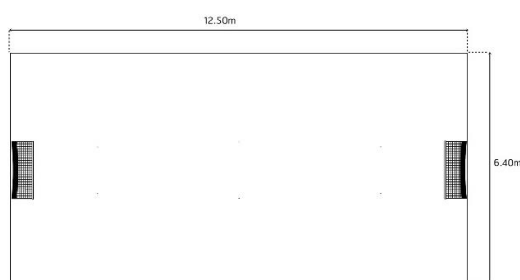


**Figure 20.** Layout in above view shows traffic route and vehicle parking area.



**Figure 21.** Layout in above view shows grocery store welfare.





**Figure 22.** Layout in above view shows Space for exercising and recreational activities (might be the same space of assemble point in emergency)

4. Compare with the result of Khantiwong, (2006) it was composed by the space and building usage of living and daily routine. While discussing and comparing with accommodation building layout in this designing, there are some agreeable with each other and not agreeable in details by Table 5.

**Table 5.** Comparing camp site for construction labors of West Construction Company (2006).

Item	Camp site for construction labor of West construction company (2006)	Temporary camp site welfare in this study
1	Pattern of temporary accommodation 1 <sup>st</sup> floor zinc row houses 1 <sup>st</sup> floor zinc row houses facing back to collide 2 <sup>nd</sup> floor zinc row house 2 <sup>nd</sup> floor zinc row house facing back to collide	Accommodation building provided the criteria as not lower than 3 m <sup>2</sup> for 1 labor.
2	Shower as public bath room with covering roof no sex separated with 0.80 meters high cement tank, size depend on number of labors, water volume calculated by personal usage in basic 20 liter/1 day/1 person.	1 wash room per labor by male and female, 1 toilet per 8 labor by male and female
3	Shower room and toilet near joint bathroom separated by zinc page in size 0.9 X 0.5 meters, 0.40 meters high water pond along the line of squatting toilet that set more when labor added.	Shower and toilet building for taking a shower excreting and washing not lower than 1 m <sup>2</sup> per 1 labor.
4	Washing space co-operate with space of joint bathroom.	Washing space as 20 % of shower and toilet building space in minimum, install washing tub with water tap that might be cement platform with putty tile.
5	Grocery store, set as not more than 2 shops	Space for welfare grocery or food shop, not lower than 0.25 m <sup>2</sup> per 1 labor.

**Table 5.** Comparing camp site for construction labors of West Construction Company (2006).

Item	Camp site for construction labor of West construction company (2006)	Temporary camp site welfare in this study
6	Activity space, empty area for sporting that composed by wile enough area.	Space for exercise, not lower than 1 m <sup>2</sup> per 1 labor.

5. Discussing with “Accommodation and labor welfare standards for employees in the category of construction business B.E. 1998”, it was able to discuss in content as follow:

In case that an employer provides accommodation for employees that should be in these options as “(1) size of room should be in narrowest as not lower than 2.50 meters, size of total space as not lower than 9 m<sup>2</sup>, and the height as not lower than 2.40 m<sup>2</sup>. Thus, there must be a space of accommodation as not lower than 3 m<sup>2</sup>/1 labor and up to building control law.” When comparing with volume of accommodation of welfare for labors providing as not lower than 3 m<sup>2</sup> / 1 labor, it is considered that size and pattern of campsite welfare in this study and in declaration of Labor Welfare Committee are agreeable to each other.

An employer should allow a provision of bathroom and toilet in these options (1) Able to separate or joint in the same room but separate male and female, designed for easy to clean and ventilator as not lower than 10 % of the space or having enough ventilation fan. The distance of vertical between ceiling or lowest partition has to be not lower than 2 meters. In case that bathroom and toilet are separated, the room size must not be lower than 1 m<sup>2</sup>. Comparing with size of shower and toilet of welfare for labors, the provision should not lower than 1 m<sup>2</sup> per 1 labor. From this, it is considered that the size and the pattern of bathroom and toilet space of campsite welfare in this study and the declaration of Labor Welfare Committee did not agree with each other.

“An employer should manage solid waste in accordance with law on public health and drainage should be proper and sufficient for not causing nuisance to others, not letting water flow to other space close to it for hygiene purposes.” In comparing with accommodation of welfare for labors that provided size of sanitation space composed as garbage gathering space as not lower than 0.10 m<sup>2</sup> per 1 labor for gathering food garbage space after meal to eliminate sanitary, the space for trash storage as not lower than 0.25 m<sup>2</sup> per 1 labor by principle of community trash to eliminate properly and space for waste water treatment as not lower than 0.50 m<sup>2</sup> per 1 labor and treat by proper process that might be activated sludge or constructed wetland. This is considered that size of

sanitation usage space of campsite welfare in this study and declaration of Labor Welfare committee is agreeable to each other.

6. Considering with “Manual of Accommodation for foreign labor standards by Provincial Labour Office Samutsakhon (2012)” it was able to discuss in content as follows:

Regarding the size of room that campsite welfare in this study provided, it is not lower than 3 m<sup>2</sup> per 1 labor, whereas the manual provided as not lower than 2.5 m<sup>2</sup> per 1 labor that might be mentioned for accommodation of welfare for labors providing for better room size. However, the minimum size of room of manual was provided as not lower than 8 m<sup>2</sup> per 1 labor that was higher.

Regarding the size of toilet and shower room that campsite welfare in this study provided, they were separated from accommodation room and did not provide reference no. of toilet and shower per accommodation room. Since temporary building is for easy to demolish, it uses the criteria no. of labor per space of toilet and washing to provide size and no. of toilet and shower. This was not agreeable with the manual that provided size as 0.90 X 0.90 meters.

7. Discussing with the provision “Environmental Health standard by this manual” campsite welfare in this study provided Sanitation usage space composed by garbage gathering 0.10 trash storage 0.25 and waste water treatment 0.50 m<sup>2</sup> per 1 labor. All mentioned agreed with provision about Environmental Health standard by this manual that provided waste water collecting, excreta containing to treat and discharge.

8. Comparing with the “Safety Health and Welfare on Construction Site A Trying Manual” by International Labor office International Labor Office Geneva (1999) that was able to discuss in topic 13, the welfare mentioned wildly about facilities for supporting the happiness and quality of life that an employer must prepare for employees. These were in Sub topic: 13.1 Why welfare facilities, 13.2 Sanitary facilities, 13.3 Washing facilities, 13.4 Facilities for supplying food and drink, and eating meals, 13.8 First aid, 13.9 Fire precautions that there were the same principle of campsite welfare in this study. However, it was able to be applied to care employee’s life in various cases.

9. Discussing with content of the Workers’ accommodation: processes and standards A guidance note” by IFC and the EBRD (1999) that there were content in PART II: that there were content in part II: standards for and management of workers’ accommodation 1. Standards for workers’ accommodation, on page 11 - 18., these were composed by details as A. National/local standards,

B. General living facilities, C. Room/dormitory facilities, D. Sanitary and toilet facilities, E. Canteen, cooking and laundry facilities, F. Standards for nutrition and food safety, G. Medical facilities and H. Leisure, social and telecommunication facilities that each sub topic mentioned to provide proper condition and area for supporting quality of life of labors like campsite welfare in this study.

10. Discussing the study of 2 accommodations for labor of 2 construction projects comparing Angsuwatcharakorn (2019), it could be discussed in details like campsite welfare in this study. Accommodation of company A provided and separated space for accommodation and facilities by utility as 14 sub-space composed by the space for accommodation building, toilet, shower and watch clothes, dry clothes, cook food, wash dish, grocery, nursery, head office, parking, guardhouse, litter, water tank and septic tank like company B but added 2 sub-areas which were cafeteria and staff office. Almost all mentioned details agreed with campsite welfare in this study in principle of space usage and different in some feature of workers’ living such as children care.

#### **4. Conclusion**

The providing of campsite welfare in this study for construction industry labors used the criteria of volume of space per labor to establish accommodation welfare for living mainly by focusing on physical minimum requirement that was enough and proper space for activities of living such as to sleep, to excrete, to shower, to wash clothes, to consume food and water. In addition, there are also activities to promote life quality in both physical and psychological. Those are recreation and safety in emergency such as evacuation provided as basic life quality in normal and happy living. These are not provision or rule in other content able to consider to choose properly in both physical economic and logistic area agreeing to provide and manage accommodation welfare such as raw material to build system of lightening drainage electric etc. these are details of implementation.

There should be a sketch or layout demonstrating this designing of temporary camp site welfare for samples and details of design to guide any construction project in the real implementation. This must provide, in details, of contents and plans to explain and show concretely that would be shown and discussed in the next article.

In conclusion, concept and content of temporary accommodation welfare of this research are able to be drafted as space plan in the Figure 2 by

supposed location of composed buildings or space in campsite.

## 5. Acknowledgements

This work was financially supported by the Research Grant of Burapha University through National Research Council of Thailand (Grant no. 58/2560). Grateful for sample group of labor in construction project who gave useful data and information for this research. Grateful for owners of construction project who permitted data and information collecting for this research.

## 6. Ethical approval

The study was approved by Burapha University Ethics Review Committee for Human Research Subjects (certified code: Hu 019-2560).

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# **The Utilization of ISO/IEC 27001:2013 as a Framework for Security Improvement in Accordance with GDPR for SMEs**

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## **Abstract**

General Data Protection Regulation (GDPR) – a regulation from European Union (EU) aims for the security of ‘Personally Identifiable Information’ (PII) of EU residents. It gives an individual a power to have control over the processing of their personal data by organizations. As it is, the regulation does refer to the information security controls needed to ensure the security of PII. In this paper, we propose an information security assessment on management of PII for Small and Medium-sized Enterprises (SMEs) by incorporating ‘ISO/IEC 27001:2013 Annex A. Reference control objective and controls.’ into the management of PII in accordance with GDPR for PII security improvement. We have determined that following the quantitative research method is appropriate as this research is aimed to determine the existence of information security controls applicable to the management of PII within the organization. A set of questions was created for interview with sampled organizations to determine the existence of information security controls according to ‘ISO/IEC 27001:2013 Annex A. Reference control objective and controls.’. Content analysis where pre-existing records and evidence will be requested and reviewed will also be applied to ensure that the information security controls is actually implemented.

It was found that in most organizations, however, there exists a good coverage of the information security controls according to ‘ISO/IEC 27001:2013 Annex A. Reference control objective and controls.’, but have difficulty providing evidence justifying the adequacy of the information security control implemented. This is mainly due to the lack of management systems to justify the adequacy of various security controls implemented in the first place.

‘ISO/IEC 27001:2013’ may be used as a framework for PII security control assessment to justify the adequacy or improve upon various security controls implemented for PII.

**Keywords:** ISO/IEC 27001:2013, GDPR, Data privacy, SMEs and Personally Identifiable Information

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## 1. Introduction

General Data Protection Regulation (GDPR) – a regulation from European Union (EU) aims for the security of personally identifiable information (PII) of EU residents. It gives an individual a power to possess control over the processing of their personal data by organizations.

GDPR is a set of regulation which defines how an organization will process PII of an EU individual person. Individual person is referred to as 'data subjects'.

GDPR regulations covers:

- "What is expected from an organization on various data processing activities within the organization."

- "Data subject rights to their own PII."

- "Jurisdiction of supervisory authority."

(Punit, 2018).

Implementing the organizational management of PII according to GDPR will provide benefits for the organizations, which includes:

- **"Providing insight of the data that exists within the company"** (Punit, 2018): Implementing GDPR regulations means you will have to create a list of personal data under the responsibilities within the organization, as well as the duration of storage, purpose of processing, and so on. This will give insight to the data under the responsibilities of the company and will benefits greatly for future investments in data analytics and information security controls.

- **"Exhibit transparency"** (Punit, 2018): Implementing GDPR regulations also means you must list all of PII you collect, specify how you will process it. If done correctly, will induce trust from customers as you are clear on what you do and why you do it.

- **"Data efficiency"** (Punit, 2018): Implementing GDPR regulations also means minimizing data collected. This can lead to immense business benefits e.g. efficient data processing and reduced data storage cost.

- **"Personal data security"** (Punit, 2018): Implementing GDPR regulations also means you have to implement various information security

controls to ensure confidentiality, integrity and availability of PII. If implemented properly, will reduce incidents of data breach, loss or destruction of PII, boosting reputation of the organization.

GDPR have requirements for information security which includes:

- Protection from unlawful / unauthorized access, loss or damage to PII.

- Protection from insiders's threat.

- Protection from external threat.

- Data breach notification.

- Demonstration of data protection.

(Díaz, 2016; The European Parliament and the Council of the European Union, 2016).

However, GDPR does not refer to information security controls needed for those requirements.

There is a standard published by International Organization for Standardization (ISO) on the management of information security, namely ISO/IEC 27001:2013 which defines the structure for information security management, risk assessment, documentations, and security controls necessary to ensure security of information under its scope of management (ISO27k Forum, 2016; Tzolov, 2018).

Within ISO/IEC 27001:2013 standard, there is a set of information security controls, specifically 'ISO/IEC 27001:2013 Annex A. Reference control objective and controls:' which outlines 114 information security controls on 14 different information security control topics which includes:

- "A.5 Information security policies."

- "A.6 Organization of information security."

- "A.7 Human resource security."

- "A.8 Asset management."

- "A.9 Access Control."

- "A.10 Cryptography."

- "A.11 Physical and environmental security."

- "A.12 Operations security."

- "A.13 Communications security."

- "A.14 System acquisition, development, and maintenance."

- "A.15 Supplier relationships."

- "A.16 Information security incident management."

- “A.17 Information security aspects of business continuity management.”
  - “A.18 Compliance.”
- (ISO Copyright Office, 2013).

In this paper, we propose an information security assessment on management of Personally Identifiable Information (PII) for Small and Medium-sized Enterprises (SMEs) (Thai Winner, 2020) by incorporating ‘ISO/IEC 27001:2013 Annex A. Reference control objective and controls.’ into the management of PII in accordance with GDPR for PII security improvement.

## 2. Research Methodology

The choice of method must be made in accordance with the problem addressed which is to identify the information security controls implemented within the organization. We have determined that following the quantitative research method is appropriate as this research is aimed to determine the existence and justification of information security controls applicable to the management of PII within the organization.

The data for this research was collected by the means of surveys. A set of questions was created for interview with sampled organizations to determine the existence of information security controls according to ‘ISO/ IEC 27001: 2013 Annex A. Reference control objective and controls.’

The participants were selected from Small and Medium-sized Enterprises (SMEs) which have a history of dealing with European customer(s) and were willing to contribute to this research by providing their representative to answer in person, a set of questions which were provided to determine the existence of the information security controls applicable, implemented and justified.

Content analysis where pre-existing records and evidence were requested and reviewed, were also applied to ensure that the information security controls are implemented and justified.

Out of 17 organizations inquired, 4 were willing to provide a representative to be interviewed for this research provided that they and their representative identities were kept confidential.

A Statement of Applicability, a document specific to ISO/ IEC 27001 which lists all information security controls within ‘ISO/ IEC 27001:2013 Annex A. Reference control objective and controls.’ and their applicability to the organization was modified to record the list of information security controls implemented within the sampled organization (Middleton-Leal, 2018).

The applicability of each information security controls for each sampled organization was determined first, based on whether the organization has an activity associated with the list of information security controls within ‘ISO/ IEC 27001: 2013 Annex A.’ or not.

Then, the number of information security controls implemented was determined. In order for the information security controls that existed within the company to qualify, said controls must:

- Be implemented fully or partially for the purpose of securing PII or activities regarding PII.
- Have reviewable records of implementation and operation dated back at least 3 months prior to the date of assessment.

Finally, the number of justified security controls (i.e. information security controls with evidence to justify the adequacy of information security controls implemented for the security of PII) were determined.

The results were separated into 3 groups:

- % Controls Applicable i.e.

$$\frac{\text{Number of Applicable Controls}}{\text{Number of ISO/IEC 27001 Controls}} \times 100\%$$

- % Controls implemented i.e.

$$\frac{\text{Number of Implemented Controls}}{\text{Number of Applicable Controls}} \times 100\%$$

And,

- % Controls Justified i.e.

$$\frac{\text{Number of Justified Controls}}{\text{Number of Applicable Controls}} \times 100\%$$



The results were represented into bar graphs of percentage for each sampled organization.

### 3. ISO/IEC 27001:2013 and GDPR

ISO/IEC 27001:2013 standard provides framework for information security management and GDPR defined PII as a critical information which need protection (Lopes, Guarda, & Oliveita, 2019).

ISO/IEC 27001:2013 could be implemented in a way that treats PII as information asset and set the framework for the implementation of security controls (Irwin, 2018).

For example, GDPR requirements for the security of PII includes 3 main topics:

- “Security of processing.”
- “Notification of a personal data breach to the supervisory authority.”
- “Communication of a personal data breach to the data subject.”

(The European Parliament and the Council of the European Union, 2016).

Each topic could be managed based on ISO/IEC 27001:2013 as described below:

- “Security of processing” (The European Parliament and the Council of the European Union, 2016): This topic deals with the implementation of information security controls appropriate to the risk associated. The risk to the privacy could be integrated with risk assessment required by ISO/IEC 27001:2013 standard to determine the appropriate level of security controls implemented.

- “Notification of a personal data breach to the supervisory authority” (The European Parliament and the Council of the European Union, 2016): This topic requires data controller to notify the supervisory authority of data breach within 72 hours of breach detection. This activity could be managed through ISO/IEC 27001:2013 Annex A.16.1 : “Management of information security incidents and improvements” which is a set of information security controls with an objective “To ensure a consistent and effective approach to the management of information security incidents, including communication on security events and weaknesses” (ISO Copyright Office, 2013). Which by incorporating PII data breach notification as part of data breach incident handling process, the organization would be able to comply with this topic of GDPR.

- “Communication of a personal data breach

to the data subject” (The European Parliament and the Council of the European Union, 2016): This topic requires that “When the personal data breach is likely to result in a high risk to the rights and freedoms of natural persons, the controller shall communicate the personal data breach to the data subject without undue delay” (The European Parliament and the Council of the European Union, 2016). This activity can also be incorporated with ISO/IEC 27001:2013 Annex A.16.1 as part of data breach incident handling process too.

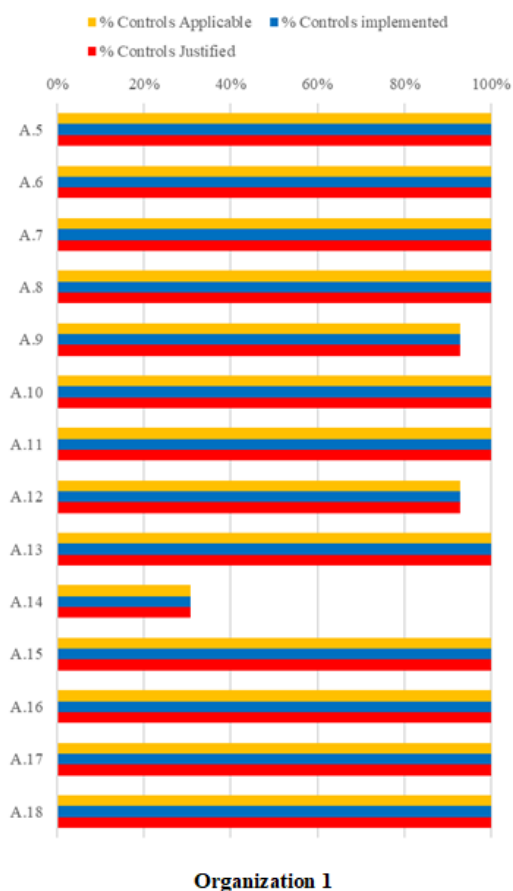
As shown above, GDPR may lack on defining the set of information security control for PII and ISO/IEC 27001:2013 could be used as a framework for information security controls implementation and management in accordance with GDPR (Clements & Milton, 2018).

### 4. Results

The result from 4 organizations were summarized into bar graphs as shown.

Organization 1: A medium sized tech company i.e. tech company with 51-200 employees with less than 200 million baht asset valuation, which has implemented ISO/IEC 27001:2013 for 1 year and was certified with ISO/IEC 27001:2013.

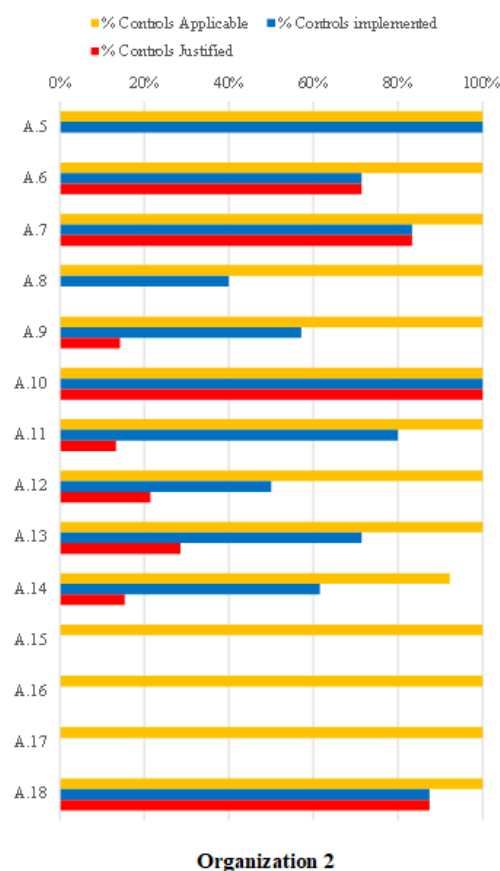
than 100 million baht asset valuation, which deals with international trading of tech commodities.



**Figure 1.** Figure showing the results from ‘Organization 1’.

‘Organization 1’ shows a strong information security controls and management implemented within the organization where all information security controls applicable were implemented and justified. The applicability and justification were supported by a risk assessment and treatment plan where PII is a part of, by being incorporated within an asset inventory which is then under the scope of each risk assessment.

Organization 2: A medium sized trading company i.e. trading company with 26-50 employees with less



**Figure 2.** Figure showing the results from ‘Organization 2’.

‘Organization 2’ shows a high coverage of applicable security controls, where existing security controls however implemented, reveals that a huge portion does not covers the aspects of the security for PII. Existing controls adequacy were only justified by contractual requirements but not based on systematic risk assessment and there’s a high risk of infringement with GDPR.

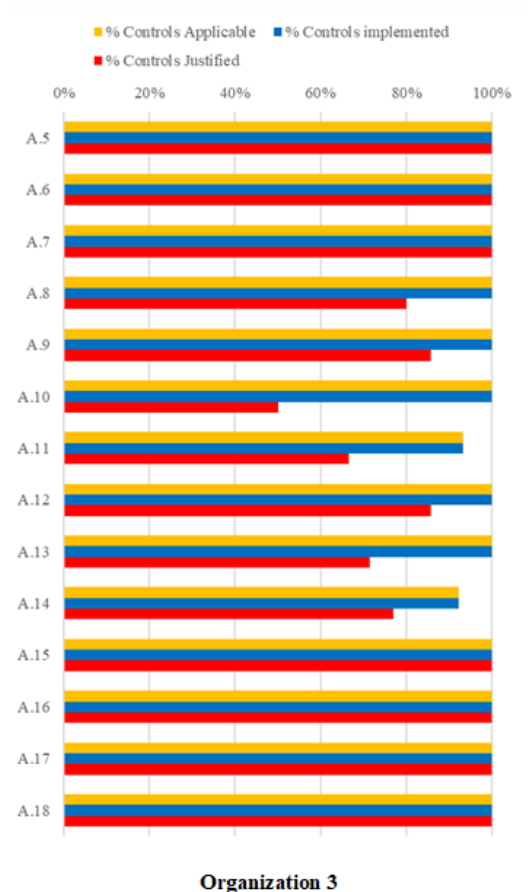
It is recommended that ‘organization 2’ should consider establishing a management system for the management of information security with regards to PII in order to plan for, implement the necessary controls, internally check for improvement and continually improve upon information security and PII management.

Organization 3: A small sized trading company i.e. trading company with less than 25 employees and less than 50 million baht asset valuation, undergoing

ISO/IEC 27001:2013 implementation. The organization has occasional EU customers.

additional aspects to the management of information security and PII management.

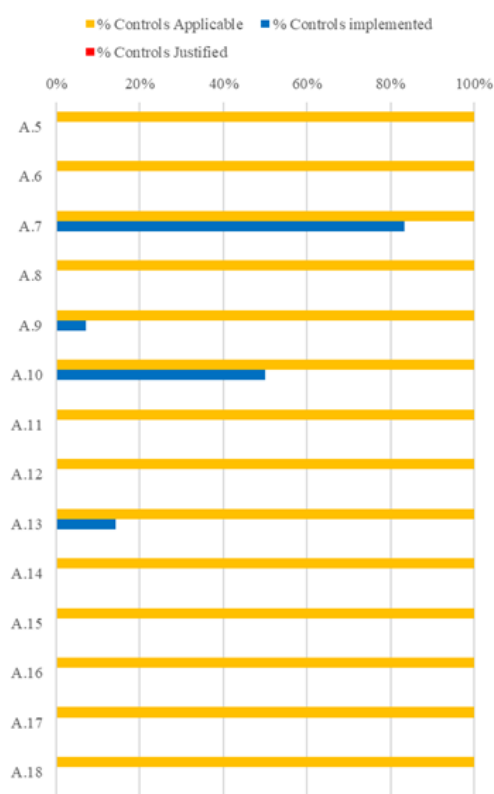
Organization 4: A small estate management company i.e. an estate management company with less than 50 employees and less than 50 million baht asset valuation, with an occasional EU client.



**Figure 3.** Figure showing the results from ‘Organization 3’.

‘Organization 3’ also shows a strong information security controls and management implemented within the organization. All information security controls applicable were implemented. The justification was a bit lacking due to the fact that risk assessment done is yet to cover the whole range of information security controls applicable. The applicability and justification were supported by a risk assessment and treatment plan where PII is a part of, by being incorporated as part of asset inventory.

As ‘Organization 3’ has an established management system in place for the management of PII, however it is lacking in some parts. It is recommended that the organization should seek expert audit or consult from 3<sup>rd</sup> party to provide



**Figure 4.** Figure showing the results from ‘Organization 4’.

‘Organization 4’ also shows a high coverage of applicable security controls. However, they are unaware of their inadequacy of information security controls that existed. The security controls for PII exists only in part. Existing implemented security controls are inevidently justifiable. GDPR infringement is certain.

It is strongly recommended that ‘organization 4’ should immediately consider to seek an expert consultation for an establishment of an information security and PII management system for the management of information security with regards to PII in order to plan for, implement the necessary controls, internally check for improvement and

continually improve upon information security and PII management.

## 5. Conclusions

It is observed that Organizations 2 and 4, while they are not implementing ISO/IEC 27001 standard, they could show the implementation and justification of the security of PII by contractual and legal means. This is in contrast with Organizations 1 and 3 which have implemented ISO/IEC 27001 as an information security framework and justify the security controls via risk assessment records. This may indicate that by setting a specific contractual or legal specification, the organization could use them as a basis for implementing and justifying information security control for PII. However, more research on this aspect may be needed.

It was found that organizations with ISO/IEC 27001:2013 implementation have less difficulty providing a good coverage of the information security controls according to 'ISO/IEC 27001:2013 Annex A. Reference control objective and controls.', on PII and have less difficulty providing evidence justifying the implementation and adequacy of the information security control for PII implemented. In contrast with an organization with unmanaged security management where they were unaware that their existing information security controls does not justifiably cover the security of PII mainly due to the lack of management systems to justify the adequacy of various security controls implemented in the first place.

'ISO/IEC 27001:2013' May be used as a framework for PII security control assessment to justify the adequacy or improve upon various security controls implemented for PII.

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# Assessment of Water Quality for Drinking and Agricultural Usages in Klong Namdang Community, Bangkok, Thailand

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## Abstract

Klong Namdang is one of the important canals in Bangkok of Thailand used as agricultural area and local lifestyle since time immemorial. Nowadays, factories are increasing around the canal that may affect to environment and human health. The objectives of this research were to study quality of consumed and canal water then assess suitability for consumption and agriculture. Therefore, this work focused on determination of physical, biological and chemical qualities of 21 drinking water samples, namely tap water, rainwater and bottled drinking water from 21 households, and 6 canal water samples from Klong Namdang community. It was found that total coliform bacteria of 27% of tap water, 100% of rainwater and bottled drinking water were out of standard quality of drinking water with  $<3-2,400$ ,  $347.50 \pm 434.38$  and  $1,204.50 \pm 1,690.69$  most probable number/100 ml, respectively. There were very strong correlations among electrical conductivity, hardness, calcium, magnesium, and sodium in drinking water with probability value  $<0.001$ . However, chemical qualities of canal water comprising magnesium, sodium, potassium, and manganese were out of standard of quality for agricultural water. Moreover, a correlation between total coliform bacteria and fecal coliform bacteria in the canal water was found with correlation coefficient of 0.9971 at probability value  $<0.001$ . Besides, water quality index of the canal was  $53.83 \pm 2.86$  meaning that it was type 4 surface water and may not be suitable for agriculture.

**Keywords:** Water quality, Drinking, Agriculture, Namdang

## 1. Introduction

Nowadays, increase of world population, technological advance, and industrial extension are main factors of worldwide environment pollution, especially water pollution. In Thailand, people use several types of water such as rainwater, bottled drinking water, tap water, surface and ground drinking water for 34.6%, 31.6%, 23.9 and 10%, respectively (Phakham, Wilachai, Silprasit, & Thummajitsakul, 2016). However, it has been reported that surface and ground water are deteriorated by salinity, nitrate, coliform bacteria and volatile organic compounds (Polprasert, 2007). Heavy metals and other pollutants in water can be also absorbed by plants (Khamson & Chirak, 2013) and transferred to consumers via food chains (Shan et al., 2013). Therefore, deteriorated water can affect to the environment (Wang, Chen, & Xia, 2010) human health (Phakhaem et al., 2016) and agriculture (Nateewattana et al., 2014). Various evidences on

effects of contaminated water have been reported previously. Deteriorated chemical water quality of Bohai Sea, China showed environmental risks (Wang et al., 2010). Misleading water consumption behavior of a community in Nakhonnayok province, Thailand caused waterborne diseases (Phakhaem et al., 2016). Moreover, use of deteriorated agricultural water showed negative effects on ion toxicity, and growth of plants (Khamson & Chirak, 2013). Klong Namdang is one of the important canals of Bangkok, capital city of Thailand, and has been used for agriculture such as flowers and fruits production. Development and growth of the city (i.e. the presence of many industrial factories) lead to water pollution, and can affect to health of the people living in the city around the canal. The objectives of this research were to study quality of consumed and canal water then assess suitability for consumption and agriculture. Thus, this work aims to identify water qualities from the community and assess the suitability of tap water, rainwater, and bottled

drinking water for drinking. The suitability of canal water for agriculture was also determined. The data gained from this work might be used for raising awareness and surveillance of waterborne diseases for the people living nearby the Klong Namdang canal.

## 2. Materials and Methods

### 2.1 Water samples

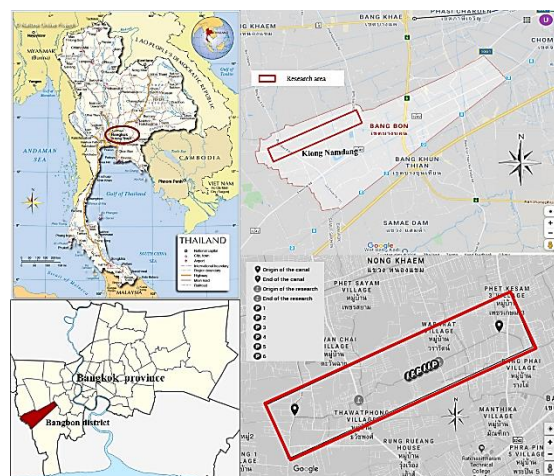
Klong Namdang canal is inhomogeneous. The canal width is 5-10 m and the length is 5.41 km with around 40 households. The area of interest is the location of agriculture, housing estates, a large drain and factories located between Bangbon 4 and Bangbon 5 Road with a length of 2.03 km and 21 households as shown in Figure 1. Six canal water samples were randomly collected from W/4, W/2, 3W/4 at 0.2D and 0.8D from 6 points in areas of agriculture, housing estates, a large drain, and factories, where W and D were width (5 m) and depth (3 m) of the canal, respectively (Maimunkongsook, 2017). Each drinking water sample was collected from 21 households in Klong Namdang community. However, sampling of drinking water was varied by water sources. All samples (1.5 l) were collected on February 11, 2018 in a polyethylene bottle and kept on ice.

### 2.2 Water quality analysis

Physical qualities (electrical conductivity (EC), and total dissolved solid (TDS), biological qualities (total coliform bacteria (TCB), fecal coliform bacteria (FCB), and chemical qualities (dissolved oxygen (DO), pH, biochemical oxygen demand (BOD), hardness (HN), and 14 metals including calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), chromium (Cr), nickel (Ni), lead (Pb), and zinc (Zn) in the canal and drinking water samples were analyzed.

Water samples were divided into 3 parts. Firstly, DO was measured by DO meter (DO-31P), and kept for physical quality analyses. EC and TDS were analyzed by laboratory method 2510 B. and 2540 C., respectively (Rice, Baird, Eaton, & Clesceri, 2012). Chemical qualities viz. pH, HN and BOD were analyzed by Laboratory of Environmental Research Institute Chulalongkorn University (ERIC) using electrometric method 4500-H+ B., EDTA titrimetric method 2340 C., and 5-days BOD test 5210 B. (Rice et al., 2012). Secondly, pH of each sample was adjusted to < 2 and 14 metals were analyzed by the ERIC laboratory. The Cr content was measured in the form of Cr<sup>6+</sup> (National Environment Board, 1994) with colorimetric method

3500-Cr D. (Rice et al., 2012) at the minimum detection limit of 0.01 mg/l.



**Figure 1.** Research area of Klong Namdang in Bangbon district in Bangkok province, Thailand.

The content of Ca, Mg, Na, and K were analyzed by atomic absorption spectrometric method 3500 B. (3111 B.) (Rice et al., 2012) at the minimum detection limit of 0.1 mg/l. Other comprising 9 metals i.e. As, Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn were assessed by inductively couple plasma method using optical emission spectrometry (ICP-OES) (Rice et al., 2012) at the minimum detection limit of 0.005 mg/l. Finally, water samples were put into sterilized glass bottles, and kept at 4°C for TCB and FCB analyses using total coliform procedure 9221 E. and *Escherichia coli* procedure 9221 F. (Rice et al., 2012) at the minimum detection limit of 3 MPN/100 ml.

### 2.3 Statistical analysis

Analysis of variance (ANOVA) in completely randomized design (CRD) used for water sampling with no replication mean comparison by Fisher's least significant difference (LSD) among types of drinking water, and Pearson's correlation were calculated by R program (R 3.5.0) for water quality factors.

### 2.4 Environmental risk quotient (RQ)

The equation (1) was used for calculating RQ from values of measured environment concentration (MEC) of Cu, Zn, Ni, Pb and Cd and predicted no effect concentration (PNEC) (Nateewattana et al., 2015) where PNEC of Cu, Zn, Ni, Pb, and Cd were 1.0, 1.7, 0.4, 1.2, and 1 µg/l, respectively (Kallqvist, 2007). RQ < 0.1 means low risk, RQ < 1.0 means moderate risk, RQ > 1 means high risk, and RQ > 10 means very high risk.

$$RQ = MEC/PNEC \quad (1)$$



## 2.5 Health risk index (HRI)

The equation (2) was used for calculating HRI. The metal reference dose (RfD) (Nateewattana et al., 2015) and chronic daily intake (CDI) were calculated from heavy metal contents, average daily drinking water, and average weight.  $HRI > 1$  means high risk for the health to have various diseases, with an exception for cancer.

$$HRI = CDI/RfD \quad (2)$$

## 2.6 Water quality index (WQI)

Some qualities of the canal water were selected to calculate WQI using the equation (3), where pH, DO, BOD, FCB, and TDS were weighted to be sub-WQI (Thongthammachart, 1997). The WQI can be used to identify types of surface water (Thongthammachart, 1997) that were classified into 5 levels, namely very good, good, moderate, poor, and very poor according to WQI ranges of 91-100, 71-90, 61-70, 31-60, and 0-30, respectively. The WQI is commonly applied to evaluate the suitability of agricultural usage of the canal water (National Environment Board, 1994).

$$WQI = ((pH)(DO)(BOD)(FCB)(TDS))^{0.2} \quad (3)$$

## 3. Results and Discussions

### 3.1 Suitability for drinking

The results showed significant differences among physical qualities of drinking water i.e. tap water, rainwater, and bottled water groups ( $P$ -value  $< 0.01$ , Table 1) and that electrical conductivity (EC) and total dissolved solid (TDS) in tap water were higher than those of bottled water and rainwater. Each average of EC and TDS was ranged in standard quality of drinking water of EC ( $< 1,500 \mu\text{s/cm}$ ) and TDS ( $< 500 \text{ mg/l}$ ), respectively. The results indicated that ions and dissolved solid contents were in acceptable range for consumption similar to a report on risk assessment of water quality for drinking of King Initiation Royal Project at Mae Pok reservoir, Lamphun, Thailand (Nateewattana et al., 2015) and Mae Puem reservoir, Phayao, Thailand (Nateewattana et al., 2014).

Each average of total coliform bacteria (TCB) from rainwater and bottled water were  $347.50 \pm 434.88$  and  $1204.50 \pm 1690.69$  MPN/100 ml, and those of tap water from selected households were in the range of  $< 3$ - 2400 MPN/ 100 ml, indicating that they were out of the standard.

**Table 1.** Quality of drinking water compared with standard of water quality for drinking.

Qualities	Unit	Mean <sup>a</sup> or Range			F <sup>b</sup>	Standard <sup>c</sup>
		Tap water	Rainwater	Bottled water		
EC	$\mu\text{s/cm}$	190.00a $\pm$ 13.07	71.83b $\pm$ 58.38	96.15b $\pm$ 128.48	16.29**	$< 1,500$
TDS	mg/l	110.53a $\pm$ 21.68	41.00b $\pm$ 38.56	52.50b $\pm$ 61.52	11.34**	$< 500$
TCB	MPN/100 ml	<b><math>&lt; 3</math>-2400</b>	<b>347.50<math>\pm</math>434.88</b>	<b>1204.50<math>\pm</math>1690.69</b>	-	$< 1.8$
FCB	MPN/100 ml	<b><math>&lt; 3</math>-23</b>	<b><math>&lt; 3</math>-4</b>	<b><math>&lt; 3</math>-4</b>	-	$< 1.8$
DO	mg/l	5.88 $\pm$ 1.56	6.06 $\pm$ 0.91	7.28 $\pm$ 0.13	0.84ns	$> 3$
pH	-	7.61 $\pm$ 0.31	7.43 $\pm$ 0.72	7.30 $\pm$ 0.42	0.68ns	6.5-9.0
BOD	mg/l	0.72 $\pm$ 0.29	0.97 $\pm$ 0.15	0.81 $\pm$ 0.32	1.32ns	-
HN	mg/l CaCO <sub>3</sub>	103.32a $\pm$ 7.93	39.85b $\pm$ 31.66	50.80b $\pm$ 69.02	16.12**	$< 150$
Ca	mg/l	28.46a $\pm$ 2.98	14.34b $\pm$ 9.64	16.77ab $\pm$ 17.01	9.94**	$< 300$
Mg	mg/l	11.85a $\pm$ 1.75	0.94b $\pm$ 0.74	6.01b $\pm$ 7.77	34.25**	$< 300$
Na	mg/l	4.68 $\pm$ 6.05	0.97 $\pm$ 0.37	2.01 $\pm$ 0.88	0.88ns	$< 200$
K	mg/l	1.08 $\pm$ 0.28	$< 0.1$ -0.84	BDL	-	$< 411$
Fe	mg/l	$< 0.005$ -0.16	$< 0.005$ -0.15	BDL	-	$< 0.3$
Mn	mg/l	BDL	BDL	BDL	-	$< 0.4$
Cu	mg/l	BDL	BDL	BDL	-	$< 2$
Zn	mg/l	$< 0.005$ -0.22	$< 0.005$ -0.23	BDL	-	$< 3$
Ni	mg/l	BDL	BDL	BDL	-	$< 0.07$
Co	mg/l	BDL	BDL	BDL	-	$< 0.011$
Pb	mg/l	BDL	BDL	BDL	-	$< 0.01$
Cr	mg/l	BDL	BDL	BDL	-	$< 0.05$
Cd	mg/l	BDL	BDL	BDL	-	$< 0.003$
As	mg/l	BDL	BDL	BDL	-	$< 0.01$

<sup>a</sup>Letter a and b are from mean comparison by Fisher's least significant difference (LSD) and BDL means below detection limit.

<sup>b</sup>Statistic F from analysis of variance (ANOVA), and ns and \*\* are non-significant difference and highly significant difference at  $p < 0.01$ , respectively.

<sup>c</sup>Standard quality of drinking water created by World Health Organization (WHO) (Nateewattana et al., 2015).

TCB of tap water samples was 27% over the standard, and below the minimum detection limit by 73%, while rainwater and bottled water samples exceeded their standard values by 100% (data not shown). These values showed that the water used for consumption in this community was contaminated with coliform bacteria and may cause health effects to the people. The bacterial contamination in rainwater can arise from insufficient cleaning of the water in the selected households. Furthermore, use of reused bottles may also cause the contamination in the bottled drinking waters. To reduce the risks of the contamination, water filtration might be employed to remove the bacteria as shown in previous report that tap water filtrated by ceramic filtering showed the TCB and FCB values of 1.2 and 0 MPN/100 ml, which were in standard range (Pattana & Pongsakri, 2002). Additionally, vegetative bacterial cells, fungi and virus were able to be eliminated from water by using boiling method (Suwanpinij & Suwanpinij, 2017).

Differences of dissolved oxygen (DO) and pH among 3 groups of drinking water were not found but remained in the standard values of  $>3$  mg/l and 6.5-9.0, respectively. Similar report indicated that DO of consumed water in Mae Pok reservoir, Lumpun, Thailand was in the standard (Nateewattana et al., 2015). In contrast, Nimrat, Suechamnonkitchakarn, Supannapan, and Vuthiphandchai (2015) suggested that pH of 7 drinking bottled water samples from Buriram, Thailand were out of the standard. Water with high DO has been known to be appropriate for drinking and less contaminated with organic matters, which was unquestionably not a cause of water pollution (Nateewattana et al., 2014). According to a specification from United States Environmental Protection Agency (EPA), pH evaluation is a secondary drinking water standard and might not directly affect to the health (Nimrat et al., 2015). However, pH is known to be crucial for metabolisms in all organisms. It is reasonable that consuming water with pH abnormality may cause long-term health problems. In this study, differences of hardness (HN) were highly significant among 3 groups of drinking water, while the standard value was reported at  $<150$  mg/l  $\text{CaCO}_3$  (Nateewattana et al., 2015). Mean of HN of tap water was  $103.32 \pm 7.93$  mg/l  $\text{CaCO}_3$ , while that of rainwater and bottled water were  $39.85 \pm 31.66$  and  $50.80 \pm 69.02$  mg/l  $\text{CaCO}_3$ , respectively. Accordingly, rainwater and bottled water were identified as soft water, whereas tap water was identified as moderate hard water, which had no

effect for drinking but showed some effects for daily consumption, such as producing less soap bubbles and making slag in metal containers (Prasertsin, Waiyaka, Kornochalart, & Pukumpuang, 2017).

The metal elements i.e. Ca, Mg, and Na were used to calculate means of metal contents while others were under the minimum detection limit, thus they were not used for comparing 3 groups of drinking water. The differences of Ca and Mg concentrations among three groups were significant with  $P$ -value  $< 0.01$ , while that of Na was insignificant with  $P$ -value  $> 0.05$ . However, means of all 14 metal contents were in the standard quality of drinking water (Table 1).

There were highly significant correlations among EC, TDS, HN, Ca, Mg, and Na with  $P$ -value  $< 0.001$  (data not shown). EC was correlated with TDS, HN, Mg, and Na with  $r=0.9746$ ,  $0.8102$ ,  $0.8782$ , and  $0.8044$ . TDS was correlated with HN, Mg, and Na with  $r=0.7906$ ,  $0.8578$ , and  $0.8039$ , respectively. Besides, HN was correlated with Ca and Mg with  $r=0.7927$  and  $0.8663$ , respectively. This indicated that EC, TDS, and HN were associated with the three ions.

**Table 2.** Environmental risk quotient (RQ).

Water source	No.	RQ <sup>a</sup>					Note <sup>b</sup>
		Cu	Zn	Ni	Pb	Cd	
Tap	1	BDL	BDL	BDL	BDL	BDL	-
	2	BDL	<b>129</b>	BDL	BDL	BDL	*
	3	BDL	BDL	BDL	BDL	BDL	-
	4	BDL	BDL	BDL	BDL	BDL	-
	5	BDL	BDL	BDL	BDL	BDL	-
	6	BDL	BDL	BDL	BDL	BDL	-
	7	BDL	<b>65</b>	BDL	BDL	BDL	*
	8	BDL	BDL	BDL	BDL	BDL	-
	9	BDL	BDL	BDL	BDL	BDL	-
	10	BDL	BDL	BDL	BDL	BDL	-
	11	BDL	BDL	BDL	BDL	BDL	-
	12	BDL	BDL	BDL	BDL	BDL	-
	13	BDL	BDL	BDL	BDL	BDL	-
	14	BDL	BDL	BDL	BDL	BDL	-
	15	BDL	BDL	BDL	BDL	BDL	-
Rain	1	BDL	BDL	BDL	BDL	BDL	-
	2	BDL	<b>118</b>	BDL	BDL	BDL	*
	3	BDL	<b>135</b>	BDL	BDL	BDL	*
	4	BDL	BDL	BDL	BDL	BDL	-
Bottle	1	BDL	BDL	BDL	BDL	BDL	-
	2	BDL	BDL	BDL	BDL	BDL	-
Canal	1	BDL	<b>259</b>	BDL	BDL	BDL	*
	2	BDL	BDL	BDL	BDL	BDL	-
	3	BDL	BDL	BDL	BDL	BDL	-
	4	BDL	BDL	BDL	BDL	BDL	-
	5	BDL	BDL	BDL	BDL	BDL	-
	6	BDL	BDL	BDL	BDL	BDL	-

<sup>a</sup>BDL means below detection limit.

<sup>b</sup>RQ was not an exact number because of detection limit, so risk of some samples cannot be predicted.

\*Very high risk

The environmental risk quotient (RQ) of drinking water of 3 groups is shown in Table 2. Most of the metal contents showed below detection limit (BDL). It was found that RQ of Zn of rainwater collected from 2 households were 118 and 135. Moreover, those of tap water were 129 and 65, which indicated a very high risk for household usage. Zinc is considered to be relatively nontoxic, particularly if taken orally. However, low amount of zinc has been suggested to interfere with the utilization of Cu and Fe and to adversely affect HDL cholesterol concentrations (Fosmire, 1990). Moreover, toxicity symptoms such as nausea, vomiting, epigastric pain, lethargy, and fatigue could occur with extremely high zinc intakes (Fosmire, 1990). The environmental risks of this study were similar to findings on RQ of Mae Pok reservoir, Thailand (Nateewattana et al., 2015) showing the high risk of Pb and As, and RQ of Mae Puem reservoir, Thailand (Nateewattana et al., 2014) which showed the high risk of Fe and Mn.

Health risk index (HRI) was calculated from each heavy metals (Table 3). HRI of Fe and Zn from tap water and rainwater were compared between children and adults. The values were between 0.0045-0.1022, which was less than 1, meaning that this level of heavy metals does not cause health risk of diseases.

### 3.2 Suitability for agriculture

Physical qualities of canal water from Klong Namdang, such as EC and TDS were  $581.50 \pm 18.96 \mu\text{s/cm}$  and  $371.67 \pm 23.54 \text{ mg/l}$ , respectively (Table 4), which corresponded to the TDS value in the standard of water quality for agriculture ( $<2,000 \text{ mg/l}$ ). Furthermore, the suitability of EC and TDS for irrigation were less than  $700 \mu\text{s/cm}$  and  $450 \text{ mg/l}$  as specified by Food and Agriculture Organization of United Nations (FAO) (Ayers & Westcot, 1994). Thus, according to these results, the quality of water from this canal was suitable for agriculture.

Means of biological qualities are shown in Table 4. It was found that means of total coliform bacteria (TCB) and fecal coliform bacteria (FCB) were out of the standard quality of type 3 surface water, a suitable type of surface water for agriculture. Similar reports were found such as a research on 4 main canals in Bangkok, Thailand that indicated an exceeded TCB in comparison with the standard (Rojjanaburanont, 1983). and a research on Klong Ong Ang, Bangkok, Thailand, that showed exceeded TCB and FCB compared with the standard (Sompong, 2018). Besides, TCB and FCB were positively strong correlated with high significance ( $r=0.9971$ ,  $P\text{-value} < 0.001$ ), which may result from presence of fecal coliform bacteria, such as *E. coli* in this canal, thus FCB directly affected TCB. FCB and TCB might have slight effects to agricultures since there is no limitation of these values in the standard of agricultural water. However, use of the water with high TCB and FCB for agricultural processes may cause a transfer of bacterial contamination in the harvested products, which subsequently cause health problems in case of insufficient cleaning of post-harvesting or cooking process.

All chemical contents were in two standards except biological oxygen demand (BOD) which showed  $6.94 \pm 0.98 \text{ mg/l}$ , indicating that it was out of the standard quality of type 3 surface water. Additionally, Mg, Na, K, and Mn were out of the standard of water quality for agriculture. The value of BOD was similar to a study on Klong Ong Ang, which showed an exceeded BOD compared with the standard (Sompong, 2018). Moreover, metal contents were similar to the research on Mae Puem reservoir water, Thailand where exceeded K and Mn were observed (Nateewattana et al., 2014). The K is the major element for all plants, therefore an excess K does not affect to agriculture but may affect to over growth of water plants that cause water pollution.

The water quality index (WQI) value showed  $53.83 \pm 2.86$ , indicating that it was not suitable for agriculture because the value was out of the standard quality of type 3 surface water (Table 4). However, the water was in the standard quality of type 4 surface water and able to use for consuming with sterilization as well as industrial usages (National Environment Board, 1994). Similar result was found on Wat Rachatiwas canal in Bangkok, Thailand whereby WQI was 51 and the canal water was identified as type 4 surface water (Choo-in, 2012). On contrary, investigation on Klong Ong Ang has identified the canals as type 5 surface water that was suitable for only transportation (Sompong, 2018).

**Table 3.** Health risk index (HRI) of drinking water.

Water source	No.	HRI															
		Children							Adults								
		Fe	Mn	Cu	Zn	Ni	Co	Cd	As	Fe	Mn	Cu	Zn	Ni	Co	Cd	As
Tap	1	<b>0.0210</b>	BDL	BDL	BDL	BDL	BDL	BDL	BDL	<b>0.0045</b>	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	2	<b>0.0229</b>	BDL	BDL	<b>0.0210</b>	BDL	BDL	BDL	BDL	<b>0.0049</b>	BDL	BDL	<b>0.0978</b>	BDL	BDL	BDL	BDL
	3	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	4	0.0305	BDL	BDL	BDL	BDL	BDL	BDL	BDL	<b>0.0065</b>	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	5	0.0229	BDL	BDL	BDL	BDL	BDL	BDL	BDL	<b>0.0049</b>	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	6	0.0210	BDL	BDL	BDL	BDL	BDL	BDL	BDL	<b>0.0045</b>	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	7	BDL	BDL	BDL	<b>0.0105</b>	BDL	BDL	BDL	BDL	BDL	BDL	BDL	<b>0.0489</b>	BDL	BDL	BDL	BDL
	8	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	9	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	10	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	11	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	12	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	13	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	14	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	15	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Rain	1	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
	2	BDL	BDL	BDL	<b>0.0190</b>	BDL	BDL	BDL	BDL	BDL	BDL	<b>0.0889</b>	BDL	BDL	BDL	BDL	
	3	<b>0.0286</b>	BDL	BDL	<b>0.0219</b>	BDL	BDL	BDL	BDL	<b>0.0061</b>	BDL	BDL	<b>0.1022</b>	BDL	BDL	BDL	
	4	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
Bottle	1	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
	2	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	

BDL means below detection limit.

**Table 4.** Quality of canal water compared with standard of water quality for agriculture.

Qualities	Unit	Mean <sup>a</sup>	Standard water quality for	
			Agriculture <sup>b</sup>	Type 3 surface water <sup>c</sup>
EC	µs/cm	581.50±18.96	-	-
TDS	mg/l	371.67± 23.54	<2,000	-
TCB	MPN/100 ml	<b>173,150±371,188.34</b>	-	<20,000
FCB	MPN/100 ml	<b>45,900±95,476.99</b>	-	<4,000
DO	mg/l	5.30±0.56	-	>4
pH	-	7.72±0.08	6.5-8.5	5-9
BOD	mg/l	<b>6.94±0.98</b>	-	<2
HN	mg/l CaCO <sub>3</sub>	184.83±4.54	-	-
Ca	mg/l	<b>28.63±12.80</b>	<40	-
Mg	mg/l	<b>24.48±1.45</b>	<1	-
Na	mg/l	<b>46.86±4.35</b>	<40	-
K	mg/l	<b>70.78±7.10</b>	<2	-
Fe	mg/l	0.58±0.16	<5	-
Mn	mg/l	<b>0.33±0.01</b>	<0.2	<1
Cu	mg/l	BDL	<0.2	<0.1
Zn	mg/l	BDL	<2	<1
Ni	mg/l	BDL	<0.2	<0.1
Co	mg/l	BDL	<0.05	-
Pb	mg/l	BDL	<0.05	<0.05
Cr	mg/l	BDL	<0.1	<0.05
Cd	mg/l	BDL	<0.01	<0.05
As	mg/l	BDL	<0.1	<0.01
WQI	-	53.83±2.86	-	61-70

<sup>a</sup> BDL means below detection limit.

<sup>b</sup> Standard water quality for agriculture created by Food and Agriculture Organization of United Nations (FAO) (Nateewattana et al., 2015).

<sup>c</sup> Standard quality of type 3 surface water (National Environment Board, 1994).

The environmental risk quotient (RQ) of canal water from Klong Namdang is shown in Table 2. The RQ of Zn of samples from W/4 at 0.2 D was 259, which was at very high risk (RQ>10) and not suitable for agricultural usages. The high RQ of the canal water may result from various canal crooks, main drainages from communities on Bangbon 4 and 5 Roads and inhomogeneous mixing of the canal water. The results of this study were similar to a research on Bohai Sea in the North of China, which suggested that RQ of Pb and Hg were more than 1 or at high risk (Wang et al., 2010).

#### 4. Conclusion

Rainwater and bottled water in Klong Namdang community are not suitable for drinking in terms of biological quality. Moreover, environmental risk was found in Zn of tap water and rainwater. Thus, cleaning processes are necessary for these types of water. According to WQI results, Klong Namdang was identified as type 4 surface water or low water quality. In addition, some metal contents such as Mg, Na, K, and Mn in the canal were found

to exceed the maximum limit concentrations. Thus, the canal water is not suitable for agricultural usages.

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# Phytochemicals and Antioxidant Activity in Sugarcane (*Saccharum officinarum* L.) Bagasse Extracts

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## Abstract

The aim of this work was to screen the phenolic compounds and antioxidant activity of sugarcane (*Saccharum officinarum* L.) bagasse extracts. The selected sugarcane bagasse was cultivated in Maha Sarakham province. Suphanburi 50 (SP50) and 72 (SP72) and Authong 17 (AU17) cultivars were extracted with methanol and solvent evaporated using a rotary evaporator. The methanolic crude extracts were then analyzed for total phenolic (TPC), flavonoid (TFC), saponin (TSC), condensed tannin (TCT), and proanthocyanidin (TPAC) content. It was found that AU17 extract had the highest content of phytochemicals. The AU17 extract also has the highest antioxidant activities, when studied by free radical (ABTS, DPPH) scavenging activity and metal- (FRAP, CUPRAC) reducing power. TPC was positively correlated to DPPH, FRAP, and CUPRAC than that of ABTS, while TFC showed a high correlation using all the tested methods for antioxidant activity. Using HPLC, AU17 bagasse extract showed higher phytochemical contents than SP strains. The dominant substances in the sugarcane extracts were gallic acid, *p*-coumaric acid, caffeic acid, quercetin, and epicatechin. The results suggested that sugarcane bagasse is a potential source of natural phytochemicals and might be of use as a source of substances for health benefits.

**Keywords:** Phytochemical, Antioxidant, Sugarcane, Bagasse, Crude extract

## 1. Introduction

Recently, the study of substances with protective effects against reactive oxygen and free radicals has attracted increasing attention. There are several sources of both intra- and extra-cellular free radicals (Carocho & Ferreira, 2013; Lobo, Patil, Phatak, & Chandra, 2010). Free radicals can cause the onset of oxidative stress, which can result in damage to biomolecules and chronic diseases (Dalle-Donne, Rossi, Colombo, Giustarini, & Milzani, 2006). Therefore, the interest in finding antioxidants has increased greatly, especially natural products. It is well known that plants, including vegetables, fruits, herbs, and cereals, are the main sources of natural antioxidants. Generally, plants produce various secondary metabolites including phenols, flavonoids, quinines, tannins, alkaloids, saponins and sterols (Alghazeer, El-Saltani, Saleh, Al-Najjar, & Hebail, 2012). They have revealed potent efficiency against free radicals. Moreover, they also have various bioactivities, such as anti-inflammatory, antibacterial,

anticancer, reduce the risk of cardiovascular disease and diabetes (Butsat, Weerapreeyakul & Siriamornpun, 2009). Phenolic compounds in plants have been the subject of many studies for their antioxidant properties (Denev, Kratchanov, Ciz, Lojek, & Kratchanova, 2012). Many studies have demonstrated that they significantly prevent some diseases, and reduce some effects of oxidative reactions (Meng, Fang, Qin, Zhuang, & Zhang, 2012).

Sugarcane (*Saccharum officinarum* L.) is an important economic crop in many countries including Thailand. It is planted in all parts of Thailand, especially in the northeastern area. However, the main application of sugarcane is sugar production since sugarcane has a high sucrose content (17-35%). Sugarcane has also been used for ethanol production as fuel instead of petroleum. Moreover, sugarcane is composed of many types of phytochemicals (Duarte-Almeida, Negri, Salatino, de Carvalho, & Lajolo, 2007). The phytochemicals found were varied depending upon strain and geographic area in which

the crop was planted (Feng, Luo, Zhang, Zhong, & Lu, 2014; Kraphankhieo & Srihanam, 2016; Naowaset & Srihanam, 2017).

In the sugar production process, the residual after juice extraction is bagasse. This bagasse has limited application for value added productions and still remains as waste which gradually increases every year. Therefore, the authors are interested in screening the phytochemicals in bagasse extract as well as their antioxidant activity. The obtained results would be used as basic information for further studying and adding value of this waste.

## 2. Materials and Methods

### 2.1 Chemicals and reagents

Pure standards of ferulic acid, caffeic acid, *p*-coumaric acid, myricetin, quercetin and resveratrol were purchased from Fisher Scientific (New Jersey, USA). The standards of aescin, vanillin, gallic acid, (+)-catechins, (-)-epicatechin, rutin, compounds 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 2,4,6-tris(2-pyridyl)-*S*-triazine (TPTZ), Neocuproine (2,9-dimethyl-1,10-phenanthroline) and Folin-Ciocalteu's reagent were purchased from Sigma-Aldrich (USA). The organic solvents, acetic acid, methanol and acetonitrile were of HPLC grade and purchased from Merck (Darmstadt, Germany). The other chemicals and solvents used were of analytical grade and were not further purified.

### 2.2 Materials

The bagasse of 3 cultivars of sugarcane for this work was Authong 17 (AU17), Suphanburi 50 (SP50) and Suphanburi 72 (SP72). The sugarcane bagasse was dried in an oven at 60°C for 18 h, ground into small pieces and kept in a sealed bag at room temperature.

### 2.3 Preparation of crude extract

The crude extract of bagasse was extracted by methanol. Bagasse (1g) was weighed and added in a volumetric flask and then made up to 25 mL by methanol before shaking for 48 h. Each sample was extracted separately in triplicate. Solvent was then evaporated from the extracts with a rotary evaporator. The powder of extract was separated from the round bottom bottle and weighed. The exact dried weight of crude extracts was determined before adding methanol (1:10 w/v) in order to dissolve the prepared crude extract and then stored in a freezer (-20°C).

### 2.4 Total phenolic content

The total phenolic content (TPC) of the methanolic extract was determined following the method of Pastrana-Bonilla, Akoh, Sellappan, & Krewer (2003). 200 µL of the methanolic extract was mixed with 1.0 mL of 1:10 Folin-Ciocalteu reagent and 0.8 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution. The mixture solution was allowed to stand for 30 min before absorption at 765 nm was measured with a UV-Vis spectrophotometer (Cary 60, THAI UNIQUE, Thailand). Gallic acid was used as standard and results were reported as milligrams gallic acid equivalent per gram dry weight (mg GAE/g DW).

### 2.5 Total flavonoid content

The total flavonoid content (TFC) was determined following the method described by Kubola, Siriamornpun, & Meeso (2011). 500 µL of the methanolic extract was added to 200 µL of distilled water, and then 100 µL of 5% NaNO<sub>2</sub> solution was added to the mixture. 200 µL of 10% AlCl<sub>3</sub> solution was added after 6 min and then left for another 5 min before adding 500 µL of 1 M NaOH solution. After stirring and being left to stand for 15 min, the absorbance was measured by a UV-Vis spectrophotometer at 510 nm. Catechin was used as standard and results were reported as milligrams catechin equivalent per gram dry weight (mg CE/g DW).

### 2.6 Total saponin content

The total saponin content (TSC) was determined following the method of Hiai, Oura, & Nakajima (1976). Briefly, 250 µL of standard solution or methanolic extract and 250 µL of 8% vanillin-ethanol solution were mixed. 2.5 mL of concentrated H<sub>2</sub>SO<sub>4</sub> (72%) was then added to the mixture standing in an ice water bath. The mixture solution was warmed at 60°C for 15 min and then cooled to room temperature in ice-cold water. The reaction mixture was measured at 560 nm using a UV-Vis spectrophotometer against a blank. Aescin was used as standard and results were expressed as milligrams aescin equivalent per gram dry weight (mg AES/g DW).

### 2.7 Total condensed-tannins content

Total condensed-tannins content (CDT) of methanolic extracts was investigated following the modified methods of Chupin, Motillon, Charrier-El, Pizzi, & Charrier (2013). 0.5 mL of extract was mixed with 4% vanillin-methanol and 1.5 mL of 3 M HCl.

The mixture was then allowed to stand in dark at room temperature for 15 min before measuring the absorbance at 500 nm. Catechin was used as standard and results were expressed as milligrams catechin equivalent per gram dry weight (mg CE/g DW).

## 2.8 Total proanthocyanidin content

The total proanthocyanidin content (TPAC) was analyzed with the procedure of Li et al. (2006). Each 200  $\mu$ L methanolic extract solution and 1.5 mL of 4% vanillin-ethanol solution were mixed together before adding 750  $\mu$ L concentrated HCl. After 15 min, the absorbance at 500 nm was measured using a UV-Vis spectrophotometer. Catechin was used as standard and results were reported as milligrams catechin equivalent per gram dry weight (mg CE/g DW).

## 2.9 DPPH radical scavenging activity

DPPH<sup>•</sup> scavenging activity radicals of the methanolic extracts were determined according to a previously published method (Cheok, Salman, & Sulaiman, 2014). 0.5 mL of diluted methanolic extract was added to 1 mL of freshly prepared 0.1 mM DPPH in methanol solution and then incubated at room temperature in the dark for 30 min, the absorbance was detected at 517 nm using a UV-Vis spectrophotometer. The percent inhibition of the DPPH activity was calculated following equation (1).

$$\text{DPPH inhibition (\%)} = [(A_c - A_s) / A_c] \times 100 \quad (1)$$

Where  $A_c$  = absorbance of the control (blank) and  $A_s$  = the absorbance of the extract. The antioxidant activity represented via the 50% inhibition ( $IC_{50}$ ) value.

## 2.10 ABTS radical scavenging activity

The ABTS radical scavenging activity of the methanolic extract was determined following the method described previously (Pastrana-Bonilla Akoh, Sellappan, & Krewer, 2003). 7 mM 2,2'-azino-bis(3-ethylbenothiazoline-6-sulfonic acid) diammonium salt (ABTS) was mixed with 2.45 mM  $K_2S_2O_8$  solution at the ratio of 1:1 to generate ABTS<sup>•+</sup> and left to stand in dark for 16 h until the reaction was completed. The absorbance of the ABTS<sup>•+</sup> solution was adjusted by distilled water to  $0.70 \pm 0.02$  at 734 nm. The reaction between 0.5 mL of the diluted methanolic extract and 1 mL of ABTS<sup>•+</sup> solution was performed with incubation at room temperature in the dark for 6 min before measuring absorbance at 734 nm using a UV-Vis spectrophotometer. The percent inhibition of

ABTS<sup>•+</sup> scavenging activity was calculated by following equation (2).

$$\text{ABTS inhibition (\%)} = [(A_c - A_s) / A_c] \times 100 \quad (2)$$

## 2.11 Ferric reducing antioxidant power

This reducing activity of the methanolic extract was determined by the FRAP method described by Li et al. (2006). To prepare the FRAP reagent, 1.5 mL of acetate buffer (pH 3.6), 150  $\mu$ L 20 mM  $FeCl_3$  and 150  $\mu$ L 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM, HCl was mixed and warmed at 37°C. 150  $\mu$ L of methanolic extract was added to the mixture solution, and then incubated for 15 min at 37°C. The absorbance of the mixture reaction was measured at 593 nm using a UV-Vis spectrophotometer. The results were expressed as  $\mu$ mol  $Fe^{2+}$ /g DW.

## 2.12 Cupric reducing antioxidant capacity

Measurement of cupric reducing antioxidant capacity (CUPRAC) was described by Apak, Güçlü, Özyürek, & Karademir (2004). 500  $\mu$ L of  $10^{-2}$  M  $CuCl_2$  solution was mixed with 500  $\mu$ L  $7.5 \times 10^{-3}$  M neocuproine solution in ethanol and acetate buffer at pH 7.0. The methanolic extract or standard (x  $\mu$ L) and  $H_2O$  [(550 - x)  $\mu$ L] were added to the mixture solution. The absorbance was recorded at 450 nm using a UV-Vis spectrophotometer after incubation for 30 min at room temperature. The results were expressed as milligrams Trolox equivalent per gram dry weight (mg TE/g DW).

## 2.13 Identification and quantification of phenolic compounds

The phenolic constituents of methanolic extracts were distinguished by HPLC-UV system with a reversed phase column Inertsil ODS-3, C18 (4.6 x 250 mm, i.d., 5  $\mu$ m particle size) with Shimadzu LC-20AC pumps (Shimadzu Co., Kyoto, Japan), SPD-M20A and a diode array detector. The conditions used followed Kubola, Siriamornpun, & Meeso. (2011). Elution was carried out by mobile phase comprised of deionized water with acetic acid (pH 2.74) (solvent A) and acetonitrile (solvent B), at a flow rate of 0.8 mL/min. The elution was performed by gradient system between solvent A and solvent B as follows: from 0-5 min (5-9% solvent B); from 5-15 min (9% solvent B); from 15-22 min (9-11% solvent B); from 22-38 min (11-18% solvent B); from 38-43 min (18-23% solvent B); from 43-44 min (23-90% solvent B); from 44-45 min (90-80% solvent B); from 45-55 min (isocratic at 80% solvent B); from 55-60 min (80-5%

solvent B) and a re-equilibration period of 5 min with 5% solvent B used between individual runs. The column temperature was maintained at 38°C and 20 µL injection volume was adjusted. The UV-diode array detection was set at 280 nm (hydroxybenzoic acid; gallic acid, catechin, epicatechin), 320 nm (hydroxycinnamic acid; caffeic acid, *p*-coumaric acid, ferulic acid), 306 nm (stilbene; resveratrol) and 360 nm (flavonols; quercetin, rutin, myricitin). Phenolic compounds in the samples were identified by comparing their relative retention times and peak areas and UV spectra with those of authentic compounds and were detected using an external standard method.

### 2.14 Statistical analysis

All the assays were expressed as means  $\pm$  standard deviation (SD). Data analysis used the SPSS statistical software for Windows using one-way analysis of variance. The significance with  $p < 0.05$  by the Duncan test was determined. Correlations of different assays were calculated using the correlation coefficient statistical option in the Pearson test.

## 3. Results and Discussion

### 3.1 Phytochemical contents

The yields of methanolic extracts in decreasing order were SP50 ( $7.36 \pm 1.03$  %w/w) > SP72 ( $5.42 \pm$

$1.38$  % w/w) > AU17 ( $4.61 \pm 1.10$  % w/w), respectively. Table 1 shows the phytochemical content found in bagasse extracts. The results indicated that the methanolic extract of AU17 had the highest of all phytochemical contents. In AU17, TSC was the predominant substance, followed by TPC and TFC. The SP72 extract showed higher phytochemical contents than the SP50 extract, except for TSC. However, the phytochemicals found in both the SP72 and SP50 were similar amount of phytochemicals which dramatically differed from the AU17 extract. In general, phytochemicals extracted from bagasse have a lower (about 33) phytochemicals content than that found in the sugarcane (Kraphankhieo & Srihanam, 2016; Naowaset & Srihanam, 2017). However, the phytochemical content in bagasse has the same content as that found in a partially purified fraction of the sugarcane extract (Kraphankhieo & Srihanam, 2016). This was not a mistake since the types and contents of phytochemicals were varied by planted regions, climates, strain, parts of plants, harvest times, instrument analysis, solvents, methods and procedures used, diseases interface, and cultivation practices (Antoniolli, Fontana, Piccoli, & Bottini, 2015; Berli, Alonso, Bressan-Smith, & Bottini, 2012; Feng, Luo, Zhang, Zhong, & Lu, 2014; Jayaprakasha, Selvi, & Sakariah, 2003; Li, Lin, Gao, Han, & Chen, 2012).

**Table 1.** Phytochemical contents (/g DW) of methanolic extracts.

Extracts	TPC (mg GAE)	TFC (mg QE)	TSC (mg AES)	CDT (mg CE)	TPAC (mg CE)
AU17	$12.13 \pm 0.33^c$	$10.88 \pm 0.03^c$	$43.04 \pm 0.13^c$	$2.75 \pm 0.76^a$	$3.35 \pm 0.19^b$
SP50	$6.64 \pm 0.00^a$	$4.52 \pm 0.08^a$	$30.23 \pm 0.05^b$	$1.54 \pm 0.70^a$	$2.03 \pm 0.28^a$
SP72	$8.11 \pm 0.28^b$	$10.03 \pm 0.03^b$	$29.99 \pm 0.05^a$	$2.68 \pm 0.43^a$	$2.20 \pm 0.08^a$

Results are expressed as mean  $\pm$  SD of triplicate measurements. Means with different letters in the same column represent significant differences at  $p < 0.05$ . AU17, Authong 17; SP50, Suphanburi 50; SP72, Suphanburi 72; TPC, total phenolic content; TFC, total flavonoid content; TSC, total saponin content; CDT, total condensed tannin content; TPAC, total proanthocyanidin content.

### 3.2 Antioxidant activity

Antioxidants protect the body from injurious action of free radicals. No individual technique is sufficient for evaluation of antioxidant activity and various assays must be properly utilized (Frag, Abdel-Latif, Abd El Baky, & Tawfeek, 2020). Therefore, the scavenging free radicals (DPPH and ABTS assays), and metal reducing power (FRAP and CUPRAC assays) mechanism were chosen.

The antioxidant activity of the methanolic extracts is shown in Table 2. Using DPPH assay, the AU17 extract was found to have higher antioxidant activity than SP72 and SP50, respectively. However, the SP72 extract exhibited slightly higher antioxidant activity by ABTS assay than the AU17 extract. The free radical scavenging activity of all extracts had more effect on the ABTS than the DPPH radicals. The metal-reducing power activity of the AU17 extract was equally



powerful on both ferric (FRAP assay) and cupric (CUPRAC assay). In comparison with the Suphanburi cultivars, the SP72 extract showed higher metal-reducing power activity than the SP50 extract which was about 50% lower than in Au17. The tested phytochemicals showed variable antioxidation. The difference in antioxidant activity might be caused by the contents and types of phytochemicals in each bagasse cultivar (Abu Bakar, Mohamed, Rahmat, & Fry, 2009; Rice-Evans, Miller, & Paganga, 1997). Moreover, they differ vastly depending on many factors including the growing conditions, extraction process, and chemical structure (Benjakul, Kittiphattanabawon, Sumpavapol, & Maqsood, 2014; Soto-Garcia & Rosales-Castro, 2016). The flavonoids and saponin could have interacted well with  $Fe^{2+}$  via coordinate linkages (Andjelkovic et al., 2006; Moran, Klucas, Grayer, Abian, & Becana, 1997). Moreover, phenolic compounds which contain many hydroxyl groups are good antioxidants (Xia, Wu, Shi, Yang, & Zhang, 2011). These findings are in agreement with previous studies which suggested that all phenolic compounds were involved in antioxidation (Guendez, Kallithraka, Makris, & Kefalas, 2005; Katalinić et al.,

2010; Kim et al., 2006). A previous report revealed that phenolic compounds could supply H-atoms to free radicals, resulting in prevention of oxidative stress (Bendary, Francis, Ali, Sarwat, & El Hady, 2013). This stress is a major factor of various degenerative diseases (Babbar, Oberoi, & Sandhu, 2015; Farag, Abdel-Latif, Abd El Baky & Tawfeek, 2020). Zheng et al. (2017) extracted the sugarcane bagasse by various solvent systems. The hydroalcoholic extract was the best solvent to obtain the highest total phenolic and antioxidant and enzyme inhibition activities. They also concluded that sugarcane bagasse is an excellent source of natural antioxidants. This study is in agreement with Mandelli et al. (2014) who reported xylooligosaccharides and antioxidant compounds from sugarcane bagasse via enzymatic hydrolysis. They also suggested that the phenolic compounds are positively involved in the antioxidant activity. Moreover, Bian et al. (2013) reported that the xylooligosaccharides extracted from sugarcane bagasse also exhibited antioxidant activity, like the phenolic compounds.

**Table 2.** Antioxidant activity of methanolic extracts.

Extracts	DPPH	ABTS	FRAP	CUPRAC
	(IC <sub>50</sub> mg/mL)	(IC <sub>50</sub> mg/mL)	( $\mu$ M Fe <sup>2+</sup> /g DW)	(mg TE/g DW)
<b>AU17</b>	11.13 ± 0.04 <sup>a</sup>	2.49 ± 0.08 <sup>a</sup>	61.22 ± 4.61 <sup>c</sup>	5.60 ± 0.11 <sup>c</sup>
<b>SP50</b>	19.82 ± 0.09 <sup>c</sup>	3.50 ± 0.33 <sup>b</sup>	10.53 ± 1.32 <sup>a</sup>	1.19 ± 0.02 <sup>b</sup>
<b>SP72</b>	14.11 ± 0.26 <sup>b</sup>	2.39 ± 0.03 <sup>a</sup>	39.06 ± 0.76 <sup>b</sup>	3.29 ± 0.18 <sup>a</sup>

Results are expressed as mean ± SD of triplicate measurements. Means with different letters in the same column represent significant differences at  $p < 0.05$ .

### 3.3 Correlation analysis

Previous work indicated that higher total phenolic content corresponded with stronger antioxidants activities (Zheng et al., 2017). The correlation analysis was conducted among the total phenolic, flavonoid, saponin, condensed tannin and proanthocyanidin contents and the antioxidant ability and the results are shown in Table 3. The positive correlation means that a high content of the phenolic compounds resulted from high antioxidant activity (reducing power). The positive correlations from

moderate to high value ( $r = 0.636$  to  $0.965$ ) were obtained from all tested phenolic compounds and FRAP and CUPRAC assays. In contrast, negative correlations were found for the content of the phenolic compounds and IC<sub>50</sub> value (DPPH and ABTS assays). The low IC<sub>50</sub> value corresponds to high antioxidant activity. The total phenolic content exhibited significant negative correlations ( $p < 0.05$ ) with DPPH ( $r = -0.900$ ), but not to ABTS ( $r = -0.618$ ). The TPC, TFC and TPAC exhibited significant negative correlations ( $p < 0.05$ ) with DPPH ( $r = -0.900$ ), while

there was no significant correlation to ABTS, except TFC ( $r = -0.953$ ). The TSC and CDT showed high negative correlation on the DPPH assay ( $r = -0.752, -0.697$ ). The CDT showed high negative correlation on ABTS ( $r = -0.755$ ), but TPC and TPAC showed the moderate negative correlation ( $r = -0.618, -0.514$ ). In addition, significant correlations ( $r > 0.900, p < 0.05$ ) among the four antioxidant methods were also found, except for TPC with the ABTS method. The results strongly indicated that the substances in sugarcane

bagasse are chiefly responsible for its antioxidant property. The conclusion is in agreement with previous reports (Sun, Chu, Wu, & Liu 2002; Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Byrne, 2006; Wojdylo, Oszmianski, & Czemerys, 2007; Zheng et al., 2017). Thus plants with rich phenolic content can be a valuable source of antioxidants.

**Table 3.** Correlation ( $r$ ) of phytochemical contents and antioxidant activity of sugarcane bagasse crude extracts.

Factors	TPC	TFC	TSC	TPAC	CDT	DPPH	ABTS	FRAP	CUPRAC
TPC	1	.785*	.958**	.945**	.555	-.900**	-.618	.939**	.965**
TFC		1	.589	.667*	.726*	-.975**	-.953**	.940**	.908**
TSC			1	.954**	.383	-.752*	-.393	.815**	.871**
TPAC				1	.400	-.806**	-.514	.848**	.896**
CDT					1	-.697*	-.755*	.650	.636
DPPH						1	.880**	-.988**	-.977**
ABTS							1	-.823**	-.779*
FRAP								1	.991**
CUPRAC									1

\*Correlation is significant at the 0.05 level (2-tailed).

\*\*Correlation is significant at the 0.01 level (2-tailed).

### 3.4 Identification and quantification of phenolic compounds

The individual phenolic contents in the methanolic extracts are presented in Table 4. The main phenolic compounds; caffeic acid, catechin, epicatechin, gallic acid, ferulic acid, myricetin,



quercetin, *p*-coumaric acid, resveratrol, and rutin were investigated. The results revealed that the AU17 extract had the highest concentrations of phenolic compounds, following by the SP72 and SP50, respectively. Gallic acid and *p*-coumaric acid were the predominant phenolics in the AU17 extracts while gallic acid, caffeic acid, and *p*-coumaric acid were predominant phenolics in the SP72 extract. Surprisingly, two types of phenolics; gallic acid, and *p*-coumaric acid were not found in the SP50 extract. All methanolic extracts had a similar content of ferulic acid. Proanthocyanidins in the form of monomeric phenolic compounds, such as epicatechin, were the major substances in all extracts. However, catechin was not found in both Au17 and SP50 but was observed in the SP72 extract. In addition, rutin was not found in the SP50. Myricetin was found in all extracts with low content as in the case of rutin. The large content of phenolic compounds found in the methanolic extracts were in agreement with previous

reports (Perumalla & Hettiarachchy, 2011). There has been interest in the further use of mono flavonoids due to their pharmacological effect (Guendez, Kallithraka, Makris, & Kefalas, 2005). Besides phytochemical compounds, flavonols (myricetin) were found in low content and this also agreed with previous reports (Burin, Ferreira-Lima, Panceri, & Bordignon-Luiz, 2014). It was previously reported that resveratrol is found in generally low content only in fruit pulp (Yilmaz & Toledo, 2004). This was in contrast to this work due to the resveratrol being found in moderate contents in the methanolic extracts of AU17 and was equal to quercetin content in this same cultivar. Moreover, resveratrol was also found in the methanolic extracts of SP50 and SP72 in equal amounts. The results suggested that both types and contents of phytochemicals were influenced by cultivars of sugarcane.

**Table 4.** Composition and content of phenolic compound (mg/g DW) in methanolic extracts.

Phenolic compounds	AU17	SP50	SP72
Gallic acid	1.498 ± 0.002 <sup>b</sup>	ND	0.472 ± 0.017 <sup>a</sup>
Caffeic acid	0.557 ± 0.002 <sup>c</sup>	ND	0.547 ± 0.001 <sup>b</sup>
<i>p</i> -Coumaric acid	0.960 ± 0.035 <sup>c</sup>	0.460 ± 0.036 <sup>a</sup>	0.543 ± 0.034 <sup>b</sup>
Ferulic acid	0.323 ± 0.001 <sup>c</sup>	0.317 ± 0.003 <sup>b</sup>	0.306 ± 0.001 <sup>a</sup>
Resveratrol	0.432 ± 0.024 <sup>b</sup>	0.213 ± 0.006 <sup>a</sup>	0.246 ± 0.014 <sup>a</sup>
Catechin	ND	ND	0.031 ± 0.011 <sup>b</sup>
Epicatechin	0.232 ± 0.019 <sup>b</sup>	0.156 ± 0.003 <sup>a</sup>	0.158 ± 0.023 <sup>a</sup>
Quercetin	0.430 ± 0.015 <sup>a</sup>	0.464 ± 0.031 <sup>a</sup>	0.454 ± 0.005 <sup>a</sup>
Rutin	0.014 ± 0.001 <sup>b</sup>	ND	0.001 ± 0.003 <sup>a</sup>
Myricetin	0.006 ± 0.002 <sup>a</sup>	0.015 ± 0.004 <sup>b</sup>	0.018 ± 0.002 <sup>b</sup>

Results are expressed as mean ± SD of triplicate measurements. Means with different letters in the same column represent significant differences at  $p < 0.05$ . ND = not detected

#### 4. Conclusion

This work was focused on phytochemicals and antioxidant activity of methanolic extracts in bagasse from different cultivars. In general, the phytochemicals and antioxidant activity varied by the cultivar of bagasse sugarcane. The methanolic extract of the AU17 showed the highest total phytochemicals content and antioxidant activity. The phytochemical

contents found in the bagasse extracts were positively related to their antioxidant potential. The HPLC data showed that all studied bagasse cultivars contained various phenolic compounds and also strong antioxidant potential. This work suggested that bagasse is a good source of health- supplement compounds, promising antioxidant activity via free radicals scavenging and reducing power mechanisms. It would be interesting to focus more studies on the

biological activities of their individual purified extracts.

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# Amount of macronutrients in vermicompost from tissue paper waste with earthworm

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## Abstract

This research aimed to study and compare the macronutrients in vermicompost from tissue paper waste with earthworm. Two species of earthworm (*Eudrilus eugeniae* and *Pheretima peguana*) were used in this study. The experiments were set up as 4 sets and were operated in plastic tub (60 x 40 x 13 cm) for 4 weeks. During the degradation, the moisture content was controlled at 70-80% by using sprinkling water. The macronutrients including nitrogen, phosphorus and potassium were analyzed. The result showed that the macronutrients (nitrogen, phosphorus and potassium content) in vermicompost from *Eudrilus eugeniae* were 0.0161, 16.420 and 82.428 mg/kg, respectively. The macronutrients (nitrogen, phosphorus and potassium content) in vermicompost from *Pheretima peguana* were 0.0142, 14.631 and 54.123 mg/kg, respectively. In addition, the macronutrients in vermicompost from two species of earthworm were significantly different between *Eudrilus eugeniae* and *Pheretima peguana* ( $P < 0.05$ ).

**Keywords:** Tissue papers, Earthworms, *Eudrilus eugeniae*, *Pheretima peguana*, Macronutrients

## 1. Introduction

In the past 10 years (2008-2016), the amount of solid waste has increased every year. We had the amount of 27.06 million tons of solid wastes produced and increasing amount of 1.13 to 1.14 kg per person till 2016. (Pollution Control Department, 2017)

Tissue paper plays the more important role in our daily lives because it can be used for many purposes, including bathroom and on the dining table. The consumption of paper is associated with the growth rate of the population. Plants such as reeds, jute, sugarcane, cotton, cane are also used to make pulp. The chemical composition of the wood consists of three things: 1) cellulose, 2) hemicellulose, 3) lignin (Nuonphudsa, 2014).

Waste disposal technology can be divided into 3 major systems:

1. Composting system. It is the degradation of organic substances by the biological process of microorganisms as the decomposition agent to

transform into relatively stable minerals. The composting process can be divided into two processes: the aerobic decomposition process and anaerobic decomposition process.

2. The incinerator system. It is the destruction of solid waste by incineration in a properly designed and constructed incinerator with an incineration temperature of 850-1,200 degrees Celsius for almost destruction.

3. Landfill system. Sanitary Landfill is the disposal of solid waste by bringing it to a landfill in the area provided, which is an area that has been selected according to the rational principles. Each waste disposal technology has different advantages and disadvantages. (Pollution Control Department, 2004)

Earthworms are animals that are common in the soil. They live in compost piles, under piles of manure, and in areas with sufficient sources of food and moisture. Earthworms are divided into two groups: red earthworms and gray earthworms. They

are very suitable for decomposing waste and food waste. Gray earthworms live in the soil and digest little food. Earthworms allow the process of degradable and it can increase its nutritional value higher than usual. Earthworms that are effective in digesting plant or organic debris have 5 species consisting of *Eisenia foetida*, *Eudrilus eugeniae*, *Lumbricus rebellus*, *Pheretima peguana* and *Pheretima posthuma*. There are two varieties of earthworms that are commonly used to decompose organic matter for fertilizer production in Thailand: The *Pheretima* species. *Eudrilus eugeniae* and *Pheretima peguana* (Tancho, 2000).

Organic Solid Waste Disposal in nature can be degraded with the process by microorganisms and soil animals such as earthworms. Earthworm manure can be used to improve soil quality. Compost can be produced with feeding earthworms inside containers such as plastic drawers and plastic tubs. This process can reduce odors and decompose waste or manure quickly into compost.

## 2. Materials and Methods

### 2.1 Materials

(1) Two species of earthworms are *Eudrilus eugeniae* (Figure 1) and *Pheretima peguana* (Figure 2).



**Figure 1.** *Eudrilus eugeniae*.



**Figure 2.** *Pheretima peguana*.

(2) Cow dung was used as a standard background medium that served as a control.

Preparing the area for raising earthworms: Cow dung was passed through a 2 mm sieve and dried in the sun for 14 days to remove earthworms and cocoons of earthworms and other organisms. Then, soaked 1-2 nights in water, (total of 3 rounds of water irrigations). This process took around 7 days to cool the cow dung and make it suitable for earthworm living. The pH of cow dung was measured and controlled in the range of 5.0 - 8.0 by lime solution (spray). The 10 kg of cow dung was put into a plastic tub and the moisture level controlled at 70-80% by sprinkling distilled water onto the compost medium when necessary (Department of Agricultural Extension, 2006).

(3) The diameter of the plastic tub was 60 x 40 x 13 cm, and the drainage holes were 20-30.

(4) Tissue papers from Amazon Coffee Shop were prepped.

Preparation of food for feeding earthworms: The tissue papers from an Amazon Coffee Shop were collected and cut into a size of 0.5 - 1 cm and then fermented for 7 days before degradation. This is because earthworms feed on spoiled organic waste that decomposes into a liquid (Kongthong, 2018).

### 2.2 Experimental setup

There were 2 species of earthworms used in this research: *Eudrilus eugeniae* and *Pheretima peguana*, aged 30-45 days, in four replicates for 30 days.

**Set 1** 200 g *Eudrilus eugeniae* and 10 kg cow dung (Figure 3)



(1)



(2)

**Figure 3.** (1) *Eudrilus eugeniae* (2) cow dung



**Set 2** 200 g *Eudrilus eugeniae*, 10 kg cow dung and 800 g tissue paper (Figure 4)



(3) (4) (5)

**Figure 4.** (3) *Eudrilus eugeniae* (4) cow dung (5) tissue paper.

**Set 3** 200 g *Pheretima peguana* and 10 kg cow dung (Figure 5)



(6) (7)

**Figure 5.** (6) *Pheretima peguana* (7) cow dung.

**Set 4** 200 g *Pheretima peguana*, 10 kg cow dung and 800 g tissue paper (Figure 6)



(8) (9) (10)

**Figure 6.** (8) *Pheretima peguana* (9) cow dung (10) tissue paper.

During the study, no extra feed was added at any stage. The sets were covered to protect from sun light. Additionally, the degradation of tissue was observed till the final product had black, brown and light brown colors. Then, the cow dung and vermicompost was separated and everything was dried and sieved.

The macronutrients were analyzed in vermicompost from tissue paper waste with two

species of earthworms *Eudrilus eugeniae* and *Pheretima peguana* by analyzing the nitrogen, phosphorus and potassium.

### 2.3 Laboratory Analysis Methods

The samples were air-dried before control analysis of nitrogen, phosphorus and potassium. All the samples were analyzed in triplicate and the average results were used for comparisons.

Nitrogen content was analyzed by the Kjeldahl method and started from weighing 1 gram of vermicompost and putting the sample in a Kjeldahl tube. A volume of 100 ml deionized water was added. Then, 15 g of heavy potassium sulfate, 0.7 g of mercuric oxide, and 25 ml of concentrated sulfuric acid were added. Digestion at 380°C was done using a digestion machine for about 30 minutes, until it became a clear liquid and was set aside to cool. 100 ml deionized water was added. Distillation was carried out with a steam distiller and ammonia was supported by boric acid mixed with an indicator composed of a mixture of methyl red and methylene blue. Distillation was done until the volume was at least 150 ml and cooled at room temperature. Titration was carried with standard solution 0.05 normal sulfuric acid and when it reached the end point, it got a light purple color (Choo-in, 2014).

Phosphorus content was analyzed by the Bray II method and the machine used was the spectrophotometer. Approximately 1 gram of sample was weighed in a 250 ml Erlenmeyer flask, 10 ml of Bray II extract was added, shaken for 1 minute and filtered with No.5 filter paper. 10 ml of the filtered solution was pipetted into a test tube, into which 1 ml of reagent solution was added, mixed well, and set aside for 30 minutes. The absorbance was measured at the wavelength of 880 nm and the obtained absorbance was compared with the standard curve to determine the phosphorus concentration (Choo-in, 2014).

Potassium content was analyzed by the Atomic Adsorption Spectrophotometer. Approximately 2 g of sample was weighed in a 250 ml Erlenmeyer flask, then 20 ml of mixing solution was added for extraction, shaken for 30 minutes, and strained with a filter. Afterwards, the volume

was adjusted to 25 ml with deionized water in a volumetric flask. Standard solutions were prepared at concentrations such as 0.5, 1.0, 2.0 mg per liter. Content was analyzed using flame atomic absorption spectrophotometer (Choo-in, 2014).

## 2.4 Data treatment

Samples of vermicompost that were sifted between cow dung and fertilizers were collected using a basket of vermicompost to be analyzed. Nitrogen, phosphorus and potassium content in three repetitions were done in a chemical laboratory after pre-composting for 1 week. The storage period was 4 weeks.

The data were analyzed to find the means to compare the difference in the macronutrients in vermicompost from tissue paper waste with two species of earthworms *Eudrilus eugeniae* and *Pheretima peguana*.

## 2.5 Statistical analysis

The differences in macronutrients were statistically interpreted using t-test at 95% confidence. These tests were performed to find out any significant comparison between the macronutrients in vermicompost from tissue paper waste with two earthworm species. Statistical significant was at  $P < 0.05$ .

## 3. Results and Discussion

### 3.1 The macronutrient in vermicompost

The macronutrient which was nitrogen content in vermicompost from the degradation of two species of earthworms is shown in Table 1.

**Table 1.** Nitrogen content ( mg/ kg) of the vermicompost from 4 sets of experiments.

Week	<i>Eudrilus eugeniae</i>		<i>Pheretima peguana</i>	
	Cow dung	Cow dung mixed with tissue paper	Cow dung	Cow dung mixed with tissue paper
1	0.0167	0.0133	0.0130	0.0143
2	0.0163	0.0130	0.0120	0.0160
3	0.0160	0.0173	0.0130	0.0133

4	0.0157	0.0207	0.0160	0.0133
$\bar{X}$	0.0162	0.0161	0.0135	0.0142

For the degradation of *Eudrilus eugeniae*, nitrogen content in vermicompost from cow dung degradation was in the range of 0.0157-0.0167 mg/kg ( $\bar{X}$  = 0.0162) and showed the maximum value at 0.0167 mg/kg at 1 week while the degradation of cow dung mixed with tissue paper was in the range of 0.0130-0.0207 mg/kg ( $\bar{X}$  = 0.0161) and the maximum value was 0.0207 mg/kg at 4 weeks. For the degradation of *Pheretima peguana*, nitrogen content in vermicompost from cow dung degradation was in the range of 0.0120-0.0160 mg/kg ( $\bar{X}$  = 0.0135) and showed the maximum value at 0.0160 mg/kg at week 4 whereas cow dung mixed with tissue paper degradation was in the range of 0.0133-0.0160 mg/kg ( $\bar{X}$  = 0.0142) and the maximum value was 0.0160 mg/kg at 2 weeks. Phosphorus content in vermicompost from the degradation of two species of earthworms is shown in Table 2.

**Table 2.** Phosphorus content ( mg/ kg) of the vermicompost from 4 sets of experiments.

Week	<i>Eudrilus eugeniae</i>		<i>Pheretima peguana</i>	
	Cow dung	Cow dung mixed with tissue paper	Cow dung	Cow dung mixed with tissue paper
1	13.606	17.188	13.326	13.924
2	13.361	17.378	13.924	14.324
3	17.644	16.478	13.490	14.825
4	20.687	19.306	14.920	15.451
$\bar{X}$	16.325	17.588	13.915	14.631

Table 2 shows the phosphorus content in vermicompost by degradation with *Eudrilus eugeniae*. Cow dung degradation was in the range of 13.361-20.687 mg/kg ( $\bar{X}$  = 16.325) and showed the maximum value at 20.687 mg/kg at 4 weeks while the degradation of cow dung mixed with tissue paper was in the range of 16.478-19.306 mg/kg ( $\bar{X}$  = 17.588) and the maximum value was 19.306 mg/kg

at 4 weeks. For the degradation of *Pheretima peguana*, phosphorus content in vermicompost from cow dung degradation was in the range of 13.326-14.920 mg/kg ( $\bar{X}$  = 13.915) and showed the maximum value at 14.920 mg/kg at 4 weeks whereas cow dung mixed with tissue paper degradation was in the range of 13.924-15.451 mg/kg ( $\bar{X}$  = 14.631) and the maximum value was at 15.451 mg/kg at 4 weeks.

Potassium content in vermicompost from the degradation of two species of earthworms is shown in Table 3.

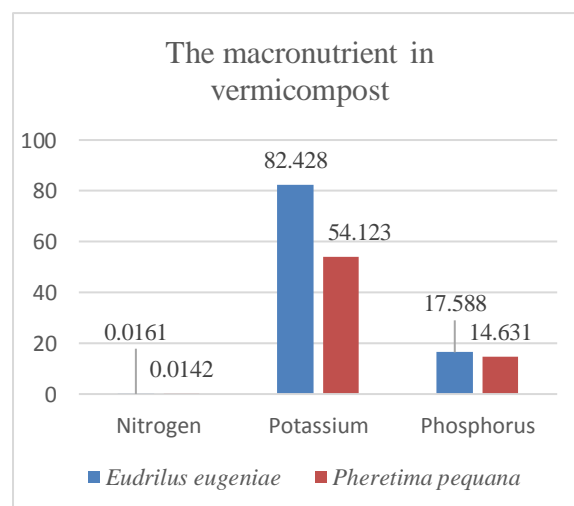
**Table 3.** Potassium content (mg/kg) of the vermicompost from 4 sets of experiments.

Week	<i>Eudrilus eugeniae</i>		<i>Pheretima peguana</i>	
	Cow dung	Cow dung mixed with tissue paper	Cow dung	Cow dung mixed with tissue paper
1	86.870	94.798	46.317	76.699
2	67.552	79.297	37.882	42.592
3	65.782	78.824	37.775	41.476
4	68.365	76.794	46.928	55.725
$\bar{X}$	72.142	82.428	42.226	54.123

Table 3 shows the potassium content in vermicompost by degradation with *Eudrilus eugeniae*. Cow dung degradation was in the range of 65.782-86.870 mg/kg ( $\bar{X}$  = 72.142) and the maximum value was 86.870 mg/kg at week 1 while the degradation of cow dung mixed with tissue paper was in the range of 76.794-94.798 mg/kg ( $\bar{X}$  = 72.142) and the maximum value was 94.798 mg/kg at week 1. For the degradation of *Pheretima peguana*, potassium content in vermicompost from cow dung degradation was in the range of 37.775-46.928 mg/kg ( $\bar{X}$  = 42.226) and the maximum value was 46.928 mg/kg at weeks 4 weeks whereas cow dung mixed with tissue paper degradation was in the range of 41.476-76.699 mg/kg ( $\bar{X}$  = 42.226) and the maximum value was 76.699 mg/kg at week 1.

### 3.2 The comparison of the macronutrient in vermicompost from tissue paper waste degradation with two species of earthworm.

Analysis of the macronutrients in vermicompost obtained from cow dung degradation and cow dung mixed with tissue paper degradation, was carried using by analyzing the nitrogen content, potassium content, and phosphorus content. The means were obtained to compare the differences in macronutrients in vermicompost obtained from the decomposition of the tissues paper waste by the 2 species of earthworms. Nitrogen content, potassium content and phosphorus content maximums as noticed in vermicompost by *Eudrilus eugeniae* were at 0.0161, 82.428 and 16.420 mg/kg respectively and by *Pheretima peguana* were 0.0142, 54.123 and 14.631 mg/kg respectively. Statistical significance was at  $P < 0.05$  (Figure 7).



**Figure 7.** Comparison of the differences in the macronutrient in vermicompost from tissue paper waste with earthworm 2 species.

### 4. Conclusions

This study concludes that there are macronutrients in vermicompost from tissue paper waste degraded with two earthworm species for 4 weeks. Maximum nitrogen and potassium contents in vermicompost by cow dung degradation with *Eudrilus eugeniae* at week 1 were 0.0167 and 86.870 mg/kg, respectively. Maximum phosphorus content at week 4 was 20.687 mg/kg. Nitrogen content and phosphorus content in vermicompost by cow dung mixed with tissue paper degraded with *Eudrilus eugeniae* maximum at week 4 were

0.0207 and 19.306 mg/kg, respectively. Maximum potassium content at week 1 was 94.798 mg/kg. Nitrogen content, phosphorus content and potassium content by cow dung degradation with *Pheretima pequana* had maximum values at week 4, which were 0.0160, 14.920 and 46.928 mg/kg respectively. Nitrogen content by cow dung mixed with tissue paper degraded with *Pheretima pequana* had a maximum at week 2 of 0.0160 mg/kg. Maximum phosphorus content at week 4 was 15.451 mg/kg. Potassium maximum content at week 1 was 76.699 mg/kg.

The mean was obtained to compare the differences in macronutrients in vermicompost obtained from the decomposition of the tissue paper waste by the two species of earthworms. Nitrogen content, potassium content and phosphorus content with maximums as noticed in vermicompost by *Eudrilus eugeniae* were 0.0161, 82.428 and 16.420 mg/kg respectively and *Pheretima pequana* were 0.0142, 54.123 and 14.631 mg/kg respectively. Statistical significance was at  $P < 0.05$ .

## 5. Recommendations

Integrated waste management systems should be developed and promoted. This is to enable the local community to eliminate waste completely and sustainably. Promoting knowledge of *Eudrilus eugeniae* earthworms can help generate additional income. Also, it can be used to manage household waste and earthworm manure in local communities.

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