SSSTJ VOL.10 NO.1 Jan 2023
ISSN 2351-0889

SSSTJ

Suan Sunandha Science and Technology Journal
Volume 10, Number 1, Jan 2023

Suan Sunandha Science and Technology Journal

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SSSTJ has both online and printing versions.
ISSN 2351-0889 (Print)
e-ISSN 2539-5742 (Online)
Published by: Faculty of Science and Technology, Suan Sunandha Rajabhat University

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Suan Sunandha Rajabhat University, Faculty of Science and Technology
1 U-thong Nok Road, Dusit, Bangkok 10300 THAILAND

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Improvement of physical and thermal properties of polyvinyl butyral coating with nanosilica

Nguyen Trung Thanh*

Department of Chemical Technology, Institute of Technology
Hanoi, Vietnam
Corresponding author e-mail: nguyentrungthanhk42@gmail.com

Received: *20 January 2022* / **Revised**: *26 May 2022* / **Accepted**: *19 July 2022*

Abstract

In this paper, polyvinyl butyral (PVB) coatings with different nanosilica contents (0- 1.5 weight percent (wt%) were prepared for improving properties such as mechanical properties, corrosion protection, thermal oxidation stability of coating. Presence of nanosilica in coating was characterized by FT-IR. Corrosion resistance of PVB coatings containing nanosilica was investigated by salt mist testing. Effects of nanosilica on adhesion, flexural strength and relative hardness and thermal oxidation stability of PVB coatings were also examined. Nanosilica with content of 1.2 wt% significantly improved mechanical properties, corrosion resistance and thermal oxidation stability of PVB coatings.

Keywords: Coating for aluminum, corrosion protection, nanosilica, polyvinylbutyral coating, paint, polymer.

1. Introduction

Polyvinyl butyral (PVB) can be used as a binder for wash primers, stoving enamels, varnishes and lacquers for different substrates. PVB coating is tough, flexible, high impact resistance, transparent and weather resistant (Rodger, 2007; Arthur, 2006). It has a very good adhesion to metals, plastics and leather as well, but the most important application of PVB in paint is wash primer for aluminum, aluminum alloy (Rike, Carsten, Thomas, Boris., & Anne, 2021; Agnieszka & Krzysztof, 2016; Zhu, Ma, Sun & Zhang, 2019; Arieh, 2008). Although it is a thermoplastic, PVB itself contains functional groups so it can participate in curing with other resins as phenolic, epoxy, nitrocellulose for making paints (Hideki & Yuya, 2021; Edwin & Agnieszka, 2011; Manjunatha, Taylor, Kinloch & Stephan, 2009; Motawie, Badr, Amer, Moustafa & Ali, 2008; Yinze, Zhaolei, Liang, Yu & Yanmin, 2017; Saad, Najat & Adnan, 2010) studied PVB blended epoxy with a weight ratio of 40 wt% epoxy resin and 60 wt% PVB at about 160 °C in 30 minutes for improving mechanical properties of PVB coating. (Guiyu et al., 2019) investigated on improving anticorrosion of PVB coating with graphene oxide and poly (methylhydrosiloxane) for aluminum alloy. Corrosion resistance of the coating in 3.5 wt% NaCl solution was done. The long-term immersion experiment indicated that studied coating could effectively protect the aluminum for up to 1200 hours. (Igor et al., 2016) studied the influence of potassium polytitanate on mechanical properties of PVB coating. Results showed that surface modification of potassium polytitanate with different coupling agents significantly improved PVB coating's mechanical properties as adhesion, tensile strength and strain at break.

Some authors have used nanosilica to enhance properties of coatings or polymer composite (Thanh, 2022a; Malaki, Tehrani & Hashemzadeh, 2018; Meiling et al., 2020; Tsai, Huang, & Cheng, 2011). Results showed that nanosilica had enhanced tensile strength, flexural strength and thermal oxidation resistance of nanocomposite based on epoxy. Results also showed that nanosilica had considerably enhanced tribological and mechanical properties of acrylic polyurethane coatings, etc.

Even nanosilica particles have been introduced in matrixes as epoxy, acrylic, polyurethane, etc. but hardly published papers related to PVB paints or coatings. This article will present effects of nanosilica on mechanical properties, thermal oxidation stability, salt mist stability of PVB coating so as to confirm that some properties of PVB coating

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had been improved with nanosilica. Fourier transform infrared spectroscopy (FTIR) was also used to identify the presence of nanosilica in coating. Besides that, the anticorrosion performance of coatings with and without nanosilica was investigated by scanning electron microscopy (SEM) through salt spray testing.

2. Materials and methods

2.1 Chemicals

Binders: Polyvinyl butyral, B-08HX (Chang Chung Group, Taiwan) with main specifications: Specific gravity: 1.05- 1.10, Butyral content: 76- 82 %, Hydroxyl content: 18- 21 %, Acetyl content □ 2 %, Third amine group: 5- 9. Pigments: Dichromium zinc tetraoxide and carbon black (N330): Industrial products (China). Solvents: Xylene, Ethanol, Methyl Ethyl Ketone (MEK): Industrial products (China). Filler: Talc, Industrial products (China).
Additive: Nanosilica, Sigma-Aldrich with specifications as: Fine powder, Purity: 99.8%, Average size: 12nm, Specific surface area: 175- 225 m^2/g (according to BET method). Phosphoric acid 85%: Industrial product (China). Water was use as industrial fresh water.

2.2 Paint preparation

Table 1. Composition of PVB coating.

No.	Components	Content (wt%)
	Part A	
1	Polyvinyl Butyral (B-08HX)	25
2	Dichromium zinc tetraoxide	5
3	Talc	10
4	Carbon black (N330)	1
5	Xylene	24
6	Methyl Ethyl Ketone (MEK)	20
7	Ethanol	13-15
8	Nanosilica	0-2
	Part B	
1	Phosphoric acid 85%	15
2	Ethanol	70
3	Water	15

For Part A: Raw materials were prepared as Table 1. Primary grinding: Adding 90% of xylene and all dichromium zinc tetraoxide, B-08HX, Talc, MEK, ethanol to tank to stir at 20- 40 rpm for an hour. Keep the mixture for 24 hours. Fine grinding: Grinding at speed of 1,300- 1,500 rpm, until paint fineness □ 20 µm. Preparation: Adding the rest xylene, stirring for 02 hours. Getting a paint sample for testing. Filtering - canning - storage: Using a 100 hole/mm^2 mesh to remove any coarse particles or dirt, then switch to canning for storing.

For Part B: Just pouring components together.

2.3 Sample preparation

Samples for mechanical properties measurement and salt mist testing were prepared on steel panels (ISO 1514:2016). Paint coatings were deposited on the cleaned panels by using a sprayer (4 kg/cm^2 of pressure). These coatings were dried at temperature of (25±2) ºC and humidity of (50±5) % for 7 days before testing. The thickness of dried coatings was (30±3) µm.

Samples for FTIR analysis were prepared on glass substrates with 15 µm of dried thickness. These coatings were dried at temperature of (25±2) ºC and humidity of (50±5) % for 7 days before testing.

Samples for morphology on glass substrates with 150 µm of dried thickness and then dried at temperature of (25±2) ºC and humidity of (50±5) % for 7 days before testing.

2.4 Analysis methods

Adhesion of coating determined according to ISO 2409:2013. Flexural strength of coating was determined according to ISO 1519:2011. Impact resistance of coating was determined according to ISO 6272-1:2011. Relative hardness of coating was determined according to ISO 1522: 2006. Fineness of paints was determined by ISO 1524: 2020. Drying time of coating was determined according to ISO 9117-6:2012. Infrared spectroscopy (FTIR) was done on the Fourier FTIR-8700 series converter. Thermal oxidation stability: Thermal gravimetric analysis (TGA) was analyzed by NETZSCH TG 209F1 LIBRA in air with temperature speed of 10 ºC/minute from room temperature to 600 ºC. Morphology of coating film was observed by FESEM Hitachi S4800 machine with a magnification of 5,000 times and voltage of 5 KV. Salt mist, cyclic testing (5% NaCl) was determined by IEC 60068-2-52: 2017 with 04 cycles, each cycle included 02 hours spraying (at (35 ± 2) ºC), keeping humidity condition for 7 days at (40± 2) ºC, relative humidity of (93 ± 2) %.

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3. Results and discussion

3.1 Effect of phosphoric acid content on mechanical properties of PVB coating

As coating comprises two components so the ratio of Part A and Part B will strongly affect the mechanical properties of PVB coating. By investigating the effect of Part B (phosphoric acid) content on mechanical properties of coating, the best suitable ratio of Part A and Part B would be determined. Samples were made as Part A without nanosilica and Part B content of 10; 15; 20; 25; 30 wt%. Samples were covered on standard steel panels (ISO 1514:2016) with a thickness of 30 µm. Results were shown in Table 2.

Table 2. Effect of phosphoric acid content on mechanical properties of PVB coating.

Samples	Part A/ Part B (Wt%)		Mechanical properties of coating			
	Part A	Part B	Adhesion (Points)	Flexural strength (mm)	Impact resistance (Kg.cm)	Relative hardness
M1	100	10	3	3	160	0.31
M2	100	15	2	2	180	0.45
M3	100	20	1	2	200	0.53
M4	100	25	1	2	200	0.59
M5	100	30	2	4	180	0.61

Table 2 showed that with different percentages of Part B (or phosphoric acid content), different values of coating's adhesion, flexural strength, impact resistance, and relative hardness gained. Results also showed that Part B increased from 10-25 wt%, mechanical properties changed strongly except flexural strength. It can be explained that PVB coating was formed by curing the OH group of PVB and OH group of phosphoric acid (Saad, Najat & Adnan, 2010). More Part B or more phosphoric acid meant more stereo-linkages occurred and adhesion, impact resistance, and relative hardness of coating would be improved. When content of Part B reached 30 wt%, three-dimensional network increases while adhesion, flexural strength, impact resistance, relative hardness of coating decreased because coating was harder and more brittle (Olagoke & Kolapo, 2015). Results also showed that M4 expresses the best properties of all, so this was chosen for further research.

3.2 Effect of nanosilica content to adhesion, flexural strength and relative hardness of coating

For Investigating effect of nanosilica content on adhesion, flexural strength and relative hardness of PVB coating, samples were prepared with M4 of Table 2 and nanosilica content of 0.5; 0.8; 1.2 and 1.5 wt%. Samples were named M6, M7, M8 and M9. Samples were covered on standard panels with a thickness of 30 µm. Results presented in Table 3.

Table 3. Effect of nanosilica content on adhesion, flexural strength and relative hardness of coating.

Sample	Adhesion (Points)	Flexural strength (mm)	Relative hardness
M4	1	2	0.59
M6	1	2	0.61
M7	1	2	0.62
M8	1	2	0.64
M9	3	4	0.65

Table 3 showed that nanosilica content increased from 0.5-1.2 wt% besides, relative hardness increased, while adhesion and flexural strength were the best. However, when nanosilica content reached 1.5 wt%, hardness of coating increased slightly however, adhesion and flexural strength of coating were higher. It meant that the coating was more rigid or brittle and lost its adhesion. It can be explained that nanosilica particles had high surface energy so they tended to agglomerate into larger particles to release surface energy. In this case, when content of nanosilica in paint was high enough they would be agglomerated together and adhesion beside flexural strength of coating would be decreased (Guiyu et al., 2019; Pritom, Irthasa, Vinoy, Praveen & Giridhar, 2018).

3.3 Infrared spectroscopy (FT-IR) analysis

To investigate the appearance of nanosilica in PVB coating, infrared spectroscopy (IR) of PVB coating with and without nanosilica were conducted

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by Fourier FTIR-8700 series transformers. Results presented in Table 4 and Figures 1a, 1b.

Table 4. Selected measured IR bands of coating.

No.	Typical spectrum	Wavenumbers (cm^{-1})
1	νOH	3387.85
2	νCH (Aliphatic hydrogens)	2955.89
3		2912.10
4		2869.49
5	νC=C (alkene)	1646.27
6	ν_aC-O-C (asymmetry)	1100.13
7	ν_a C-O-C (symmetry)	1049.04
8	νSi-OH	936.27
9	νSi-O-C	768.15

Figure 1a shows that (Emily, 2017) peak 3387.85 cm-1 of OH group in PVA (raw material for making PVB), peaks 2955.89, 2912.10, 2869.49 as CH group of aliphatic hydrogens, 1646.27 as C=C of alkene, 1378.51 of methyl group, 1239.68 of acetates, 1100.13, 1049.04 as -C-O-C (Symmetry) of second alcohol, 994.80 as =C-C (Bending) of trans RCH=CHR, 807.90 as CH out of plane bending. There had some new peaks on figure 1b, in which, peak 936.27 cm-1 represented for vibration of Si-OH bond, and peak 768.15 is typical for the vibration of the Si-O-C bond, which proved that there had nanosilica particles in FT-IR spectrum captured sample.

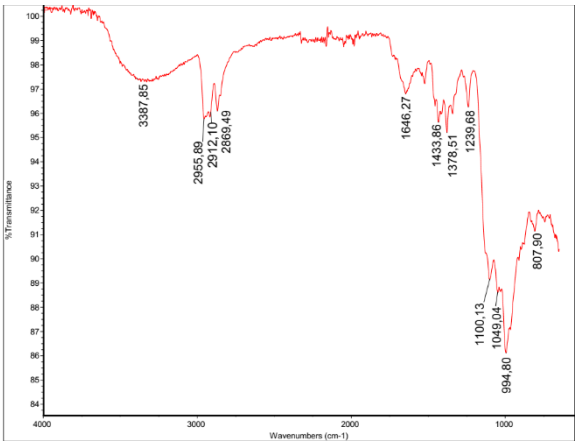

Figure 1a. IR of PVB coating.

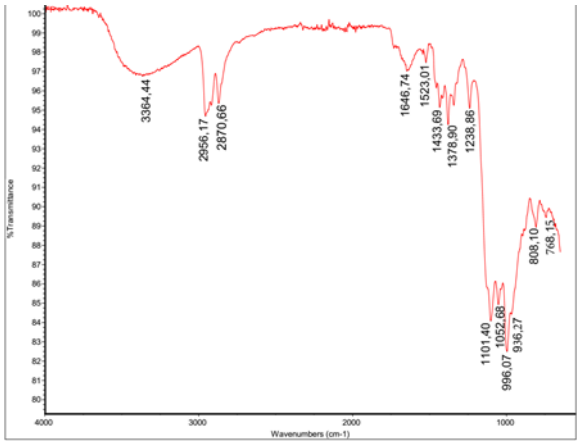

Figure 1b. IR of PVB coating with nanosilica.

3.4 Salt mist stability of PVB coating with and without nanosilica

To investigate the effect of salt mist on protection of PVB coating with and without nanosilica, samples M4 and M8 as mentioned above were conducted. Coatings were tested in a salt mist chamber with 5% NaCl for 04 cycles. Optical photographs on the surface of coatings were examined by cross-cut test (cross square on the surface of coating was scratched, cleaned carefully) before and after salt mist cyclic testing and SEM also was taken with magnification of 5,000 times to observe surfaces of coatings before and after testing. Results observed in Figures 2a, 2b, 2c, 2d and Figure 3.

Figures 2a, 2b, 2c, 2d showed that no obvious exfoliation phenomenon was observed in coatings after testing, which indicated that coating had a strong adhesion and was tightly bonded with steel substrate. Meanwhile, Figure 3 showed that after 04 cycles of salt mist testing of coatings, rust spots, PVB coating sample had larger blistering, PVB with nanosilica coating sample had some smaller spots blistering. They indicated that the steel surface under coating had been corroded and degraded adhesion. This meant that PVB coating with nanosilica could protect substrates better than PVB coating without nanosilica. This can be explained that good adhesion came from the role of nanosilica. On the surface of nanosilica, there were Si-OH groups which created physical bonding to substrate. The strong adhesion guaranteed the long-term protection of PVB with nanosilica coating. Results also indicated that salt mist testing happened electrochemically so SEM images could not clearly express the differences between samples (Hideki & Yuya, 2021; Yinze et al., 2017).

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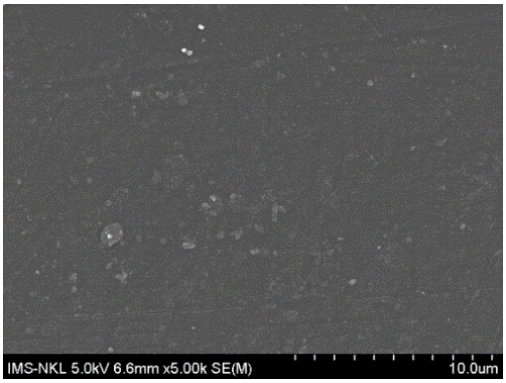

Figure 2a. SEM of M4 before salt mist testing.

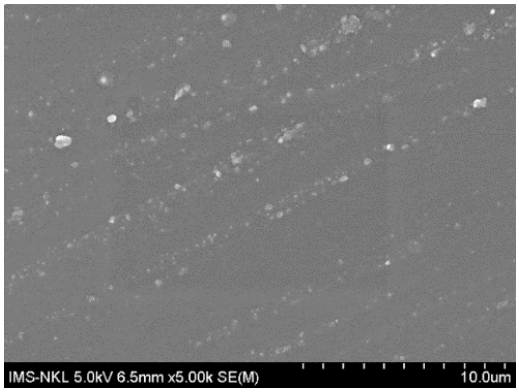

Figure 2b. SEM of M4 after salt mist testing.

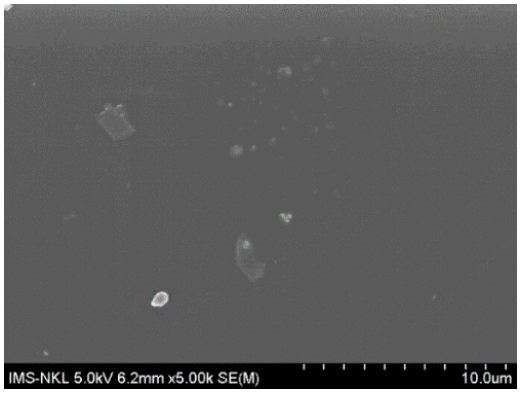

Figure 2c. SEM of M8 before salt mist testing.

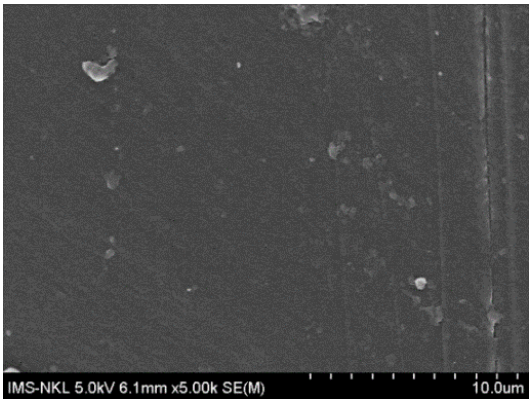

Figure 2d. SEM of M8 after salt mist testing.

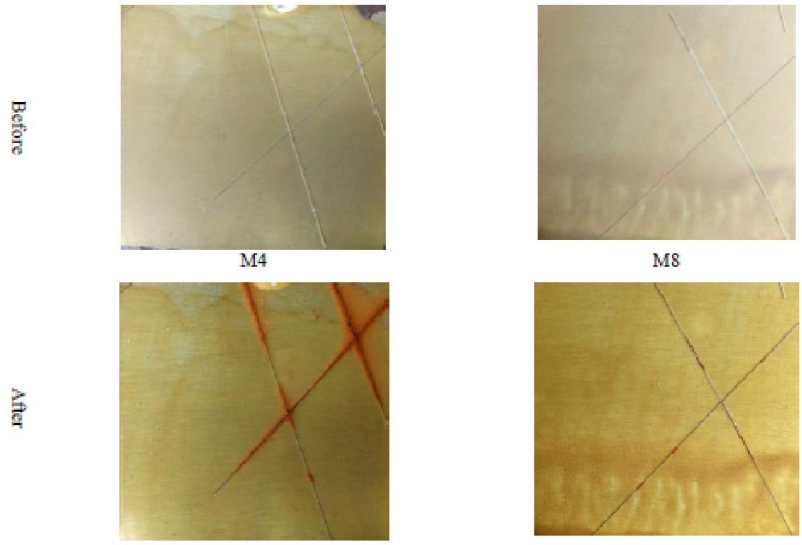

Figure 3. Optical photographs on surface of coatings examined before and after salt mist testing.

3.5 Effect of nanosilica content on thermal oxidation resistance of PVB coating

To study the effect of nanosilica content on thermal oxidation resistance of PVB coating, Thermogravimetric analysis (TGA) was done with samples M4 (PVB coating) and M8 (PVB with nanosilica coating). Table 5 and Figures 4a, 4b presented the results.

Table 5 and Figures 4a, 4b showed that the slope of TG curves of different samples were not the same. At different temperatures, decomposition of samples was different, too. At a temperature of under 200 ºC: Decomposition occurred with low molecular

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substances and residual solvents as well. From room temperature to 300 ºC: Decomposition happened with residual functional groups in polymer branches, low molecular substances, (Thanh 2020; Thanh 2022b). For the total decomposition volume from room temperature to 350 ºC, aged M4 had the highest decomposition of 45.08 %. M8 had the lowest decomposition of 42.18 %. This can be explained that nanosilica had prevented chemical bonds of polymer from breaking that led to reduce slits in material structure, hence, oxygen permeation in material would be reduced and thermal oxidation resistance of material increased (M. Malaki et al., 2018; Olagoke, & Kolapo, 2015). Up to 500 ºC and above, results also showed that PVB coating has ash content of 38.78 % and in the presence of nanosilica, ash content of coating was higher, up to 43.39 %. It can be explained that, in conditions of high temperature with presence of oxygen, polymer chains were cut and oxidation of organic substances. At that time, oxygen promoted formation of free radicals and polymers would be degraded deeply, forming lower molecular compounds containing oxygen. For PVB coating with nanosilica, nanosilica particles prevented penetration of heat and oxygen into structure of PVB, in addition, thermal decomposition of nanosilica would coke to form a stable structure like ceramic Thus, nanosilica had improved thermal oxidation resistance of PVB coating (Thanh, 2022a; De, Feng, Xu, Zheng & Xiao, 2017).

Table 5. Effect of nanosilica content on thermal oxidation resistance of PVB coating.

Samples	Weight loss (%)		
	300 ºC	350 ºC	500 ºC
M4	33.96	45.08	61.22
M8	33.07	42.18	56.61

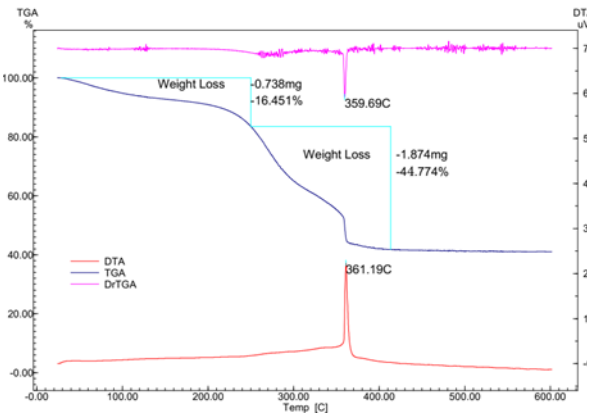

Figure 4a. TGA of M4.

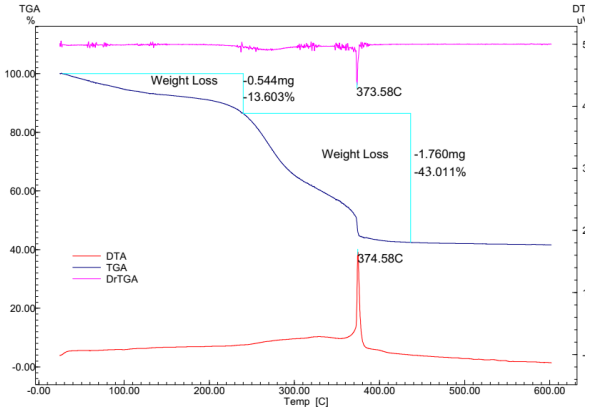

Figure 4b. TGA of M8.

4. Conclusions

- Nanosilica content increased from 0.5- 1.2 wt%, adhesion and flexural strength of PVB coating were the best, relative hardness increased from 0.59 to 0.64. nanosilica content of 1.2 wt% gave the best mechanical properties of PVB coating.

- Nanosilica improves anti-corrosion and protection of PVB coating from salt mist cyclic. PVB coating with 1.2 wt% nanosilica could protect substrate better than PVB without nanosilica.

- Nanosilica enhances thermal oxidation resistance of PVB coating. Thermal oxidation resistance of PVB coating with nanosilica is higher than that of PVB coating and ash of PVB coating is 38.78 % but of PVB coating with nanosilica is 43.39 %.

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Effects of Dried Sano (*Sesbania javanica* Miq.) Flowers Powder on the Quality of Thai Sponge Cake

Jiraporn Weenuttranon*, Patthama Hirunyophat, Nunyoung Fuengkajhornfung, Tidarat Sanphom

Department of Home Economic, Faculty of Science and Technology, Suan Sunandha Rajabhat University, U-thong Nok Road, Dusit, Bangkok 10300, Thailand

***Corresponding author e-mail: jiraporn.we@ssru.ac.th**

Received: *5 April 2022*/ **Revised**: *20 June 2022*/ **Accepted**: *30 September 2022*

Abstract

The aim of this study was to investigate the physical, nutritional values and sensory evaluation of Thai sponge cake added with different levels of dried Sano flowers powder (DSFP) as a source of carotenoids. DSFP was prepared and analyzed for total carotenoids. DSFP contained lutein, zeaxanthin and β-carotene around 29,947.94, 620.03 and 1,555.98 µg in 100 g of dry sample, respectively. The sensory liking scores were determined to be suitable to produce Thai sponge cake. Results showed that, Thai sponge cake recipe consisted of 240 g of duck eggs, 180 g of chicken eggs, 180 g of cake flour, 360 g of granulated sugar. The effects of the addition with different levels of DSFP (20, 40 and 60 %w/w) of Thai sponge cake was studied. An increase of the DSFP decreased of lightness (L*), redness (a*) value, springiness, and chewiness while yellowness (b*) value, hardness, cohesiveness, and gumminess were increased. The sensory evaluation showed that all treatments obtained the overall liking score of 6.14-6.86. The study clearly showed that the levels of 20% w/w DSFP could be a desirable amount to produce the healthy Thai sponge cake with good enough appearance, odour, taste, texture, with an overall liking score of more than 6.00 that is considered as acceptable. The developed Thai sponge cake contained protein, fat, dietary fiber, ash, moisture, and carbohydrates 9.45, 5.23, 2.64, 1.16, 33.94 and 52.42 g in 100 g respectively, and 319.10 µg/100 g β-carotene 319.10 µg/100 g. The developed product supplemented with DSFP would be a guideline to increase the health and quality of cake products.

Keywords: Thai sponge cake, Dried Sano flowers powder, Carotenoids

1. Introduction

Thai sponge cake (Kanom-sa-lee) is a Thai dish that can be categorized in the same group as western desserts, Kha-nom-farang-kudi-chin, Thai eggs cake and Kha-nom-phing. Thai dessert is unique, traditional and considered as a Thai cultural heritage (Sanphom & Weenuttranon, 2018). Thai sponge cake is a type of air-leavened cake based on wheat flour, sugar, and eggs. Cake qualities are dependent on many factors, including the ingredients used for batter preparation, aeration of batters, and processing conditions (Chaiya & Pongsawatmanit, 2011). These cakes are prepared using a combination of a batter / foam and steamed in a variety of differently shaped pans. The structure of the cake is largely determined by the amount of air in the batter (Miller, 2016). Although wheat flour is the most suitable to deliver good structure for production of acceptable cake, they are often low in nutrient density (Jonglertjesdawong, 2000). The presence of compounds of interest such as carotenoids, and phenolic compounds makes these residues attractive resources for adding agro-industrial value because they are an important source of additives for the food, cosmetic and pharmaceutical industries (Ordóñez-Santos, Esparza-Estrada, & Vanegas-Mahecha, 2021). Moreover, consumers now demand products that are free of chemical agents with health benefits; therefore, the carotenoids found in Sano flowers can serve as an alternative, natural dye in bakery products, providing antioxidant activity and provitamin A (Martins, Pinho, & Ferreira, 2017).

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Sano (*Sesbania javanica* Miq.) or Sesbania flower is a flowering plant in the Fabaceae family (commonly known as the pea family). Sano is a tropical seasonal plant which naturally grows in wet areas, especially around the ditch or canal in the central region of Thailand (Kampangkaew, Thongpin, & Santawtee, 2014). The flower is yellow and is used in several Thai recipes (Kijparkorn, Plaimast & Wangsoonoen, 2010). In terms of nutritional value, Sano is a source of vitamins, dietary fiber, calcium, and carotenoids. Biological properties of Sano have been proposed as antioxidant activity and antibacterial activity (Chakkij, 2016), which are associated with a reduction of the risk of chronic diseases (Martins et al., 2017).

Therefore, the objectives of this study were to investigate the composition of carotenoids in Sano and study the physical, chemical and sensory characteristics of Thai Sponge Cake added with different levels of dried Sano flowers powder. As well as adding value to agricultural raw materials that would be applied to the household industry level.

2. Materials and Methods

2.1 Sano flower preparation

Fresh Sano flower was bought from the local market, dried in an oven at 50ºC for 24 hours, and ground with a hammer mill (Chakki, 2016). Ground sample was analyzed for total carotenoids content by HPLC method according to Speek, Temalilwa and Schrijver (1986).

2.2 Standard recipes of Thai sponge cake

Studied the standard recipes of Thai sponge cake obtained from Siriwong (2009), Wongpaisanrit (2010) and Suban (1989) as standard recipes. The samples were evaluated by untrained panelists (n = 50) for sensory acceptability using a 9-point hedonic scale (1 = disliked extremely, 5 = neither like nor dislike, 9 = like extremely). They were instructed to first visually evaluate acceptability of product appearance, color, and then consumed each sample before scoring for odor, taste, texture and then overall liking (Meilgaard, Civille, & Carr, 1999).

2.3 Study the effects of dried Sano flowers powder on the quality of Thai sponge cake

The optimization of Thai sponge cake recipe using a 9-point hedonic scale in the previous study was selected to improve the quality of Thai sponge cake by addition of dried Sano flowers powder. All ingredients (egg, cake flour and granulated sugar) were blended with three different levels of dried Sano flowers powder (DSFP) as follows: 20, 40 and 60 %w/w. Thai sponge cake without DSFP served as the control. Ingredients were mixed by hand with a plastic scraper until smooth. The cake batter was immediately deposited into cake pans and steamed. The cakes were allowed to cool for 1 h at room temperature, after which they were removed from the pans. The cooled cakes were packed in polypropylene bags at room temperature prior to the physical, and sensory evaluation.

Color:

The color of the samples was measured using a Hunter Lab apparatus (Hunter Lab, ColorFlex, USA), which measures three parameters: L*(lightness), a*(red-green), and b*(yellow-blue).

Texture properties:

The texture of the cake samples (3×3×3 cm) were based on the hardness, springiness, chewiness, cohesiveness, and gumminess as determined using a TA.XTplus Texture Analyzer (TA.XT PlusC, Stable Micro Systems, UK), using a cylindrical-shaped probe (12.45 mm in diameter). The testing conditions were: probe distance of 50 mm, test speed of 1 mm/sec, and post-test speed of 60 mm/sec.

Sensory evaluation:

The sensory evaluation using a 9-point hedonic scale was the same procedure as described in the previous experiment .

2.4 Nutritional value of developed Thai sponge cake

The developed Thai sponge cake (from section 2.3) was determined in terms of nutritional value: moisture content, protein, fat, ash, dietary fiber according to AOAC (2019) and β-carotene content according to Speek et al. (1986).

2.5 Statistical analysis

Experimental data were carried out using the completely randomized design (CRD) in physical properties and the randomized complete block design (RCBD) in sensory evaluation. The data were analyzed using analysis of variance facilitated by the IBM SPSS® version 23 software (IBM SPSS Inc., USA). Duncan's multiple range test was used to

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determine multiple comparisons of mean values with a statistically significant difference at $p \leq 0.05$.

3. Results

3.1 Carotenoids content of dried Sano flowers powder (DSFP)

Sano flowers is an important source of carotenoid pigments, which has biological properties as antioxidant activity and antibacterial activity (Chakkij, 2016). DSFP was prepared and analyzed for total carotenoids content. DSFP contained 29,947.94 µg lutein, 620.03 µg zeaxanthin and 1,555.98 µg β-carotene in 100 g of dry sample (Table 1). This is consistent with the research of Murkovic, Mülleder and Neunteufl (2002) that studied carotenoids in different pumpkin species; it was reported that pumpkin with orange had a lot of beta carotene, and the pumpkin that was yellow in color had high lutein with antioxidant properties, furthermore Palozza, Muzzalupo, Trombino Valdannini and Picci (2006) reported that β-carotene was an initial substance in the synthesis of vitamin A and had antioxidant properties.

Table 1. Carotenoids composition of dried Sano flowers 100 g of dry sample.

Carotenoids composition	DSFP, µg/100 g
Lutein	29,947.94
Zeaxanthin	620.03
β-carotene	1,555.98

3.2 Standard recipes of Thai sponge cake

Thai sponge cake recipes showed that the three standard recipes had an effect on the appearance, odor, taste, texture and overall liking score with statistical significance ($p \leq 0.05$) but without affecting the color's preference score with statistical significance ($p > 0.05$) (Table 2).

Considering the appearance, the recipe 1 and 2 had the preference scores in the like moderately while the recipe that 3 had the like slightly due to the appearance that the recipe 3 was contained less sugar than recipes 1 and 2; it might make the Thai sponge cake look less flaky and the texture of the Thai sponge cake was dry (Figure 1). This is consistent with Chaemmek and Naiwikul (2011) reported that the amount of sugar affects the quality of the cake. The less sugar in the recipe would be the cause of cake volume reduction. The texture of the cake also would be dried and hard. The odor, taste, texture, and overall liking score shown that recipes 1 and 3 had more liking scores than recipe 2. Due to the process of making the Thai sponge cake recipe 2, there was not sifting, the flour may cause the dough to rise unevenly and the texture was tight.

Therefore, the standard recipes of the Thai sponge cake were the standard recipe 1 as the appropriate recipe for bringing to produce Thai sponge cake supplemented with DSFP. Thai sponge cake recipe consisted of 240 g of duck eggs, 180 g of chicken eggs, 180 g of cake flour, 360 g of granulated sugar.

Table 2. Sensory liking of the three standard recipes of Thai sponge cake.

Sensory characteristics	Recipes		
	1	2	3
Appearance	7.61±0.94^{a}	7.43±0.88^{a}	6.88±0.90^{b}
Color^{ns}	7.50±1.17	7.46±1.03	7.30±1.03
Odor	7.96±0.84^{a}	7.53±0.76^{b}	7.68±0.92^{ab}
Taste	7.65±1.14^{a}	7.15±1.02^{b}	7.36±1.02^{ab}
Texture	7.41±1.18^{a}	6.43±0.74^{c}	7.03±0.88^{b}
Overall liking	7.73±1.02^{a}	6.91±0.82^{c}	7.36±0.93^{b}

Mean ± SD with different lowercase superscripts in each low are significantly ($p \leq 0.05$) different; ns = not significantly ($p > 0.05$) different

Recipe 1 Recipe 2 Recipe 3

Figure 1. Characteristic of the three standard recipes of Thai sponge cake.

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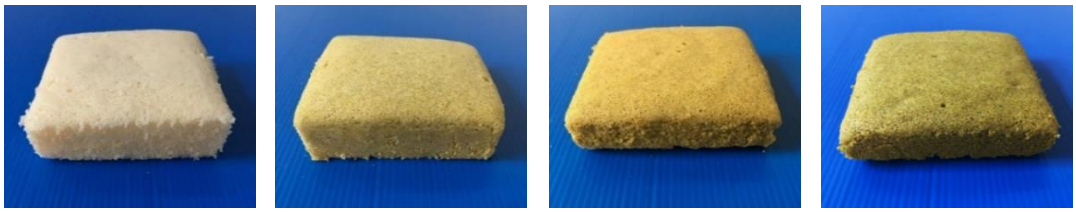
3.3 Effects of dried Sano flowers powder on the quality of Thai sponge cake

The color of the Thai sponge cake with DSFP in different levels of 20, 40 and 60 %w/w had significant effected on the lightness (L *), redness (a *) and yellowness (b *) (p□0.05) as shown in Table 3 and Figure 2. An increase of the DSFP decreased of L*, and a* value, flexibility, and cohesiveness while b* value to be increased due to the DSFP 's carotenoids, especially β-carotene. Carotenoids are among the most widely distributed pigments and naturally exhibit red, orange and yellow colors (Ngamwonglumlert & Devahastin, 2019), which affects the color of the product. This is consistent with the research of Khumkhom (2018), the color value of the Sano butter cookies has been found to decrease L*, a*, while the b* values increased when the level of DSFP increased. This made butter cookies yellow to dark brown in color. Manantaphong, Songpranam, and Dang (2011) reported that the addition of lotus (Nelumbo nucifera) in bread increased the yellow color of the bread. The texture properties of the cake products are perceptible attributes to consumers and may be correlated with the expansion and cell structure of the product. The increases in the hardness, cohesiveness, and gumminess were likely caused by decreased expansion at a higher DSFP while springiness, and chewiness to be decreased (Table 3). Sano flower has high fiber content in which the porous fibers consist of cellulose and hemicellulose can be inserted between the starch granules, that have good combination properties, resulting in decreased swelling and increased hardness of cake (Mudgil, Barak, & Khatkar, 2017).

Table 3. Physical properties of dried Sano flowers powder in Thai sponge cake.

Physical properties	DSFP (%w/w)			
	Control	20	40	60
Lightness (L*)	56.33±0.21^{a}	44.09±0.36^{b}	38.84±0.08^{c}	37.19±0.09^{d}
Redness (a*)	4.42±0.10^{a}	2.40±0.25^{d}	3.23±0.15^{c}	3.43±0.09^{b}
Yellowness (b*)	15.66±0.26^{c}	21.26±0.15^{b}	22.90±0.23^{a}	22.87±0.16^{a}
Hardness	830.65±77.54^{c}	1045.26±127.20^{cb}	1221.60±251.89^{b}	1706.39±408.59^{a}
Springiness	0.90±0.01^{a}	0.83±0.11^{ab}	0.78±0.10^{b}	0.74±0.14^{b}
Chewiness	0.84±0.01^{a}	0.81±0.02^{b}	0.78±0.04^{bc}	0.76±0.04^{c}
Cohesiveness	700.22±62.85^{c}	841.69±82.99^{ab}	943.85±157.95b	1275.98±253.47^{a}
Gumminess	633.50±62.66^{b}	705.08±142.54^{b}	753.79±213.71ab	967.69±351.42^{a}

Mean ± SD with different lowercase superscripts in each low are significantly (p □ 0.05) different



Control 20% 40% 60%

Figure 2. Characteristic of dried Sano flowers powder (control, 20%, 40%, 60% w/w) in Thai sponge cake.

The DSFP content was the most important parameter affecting the sensory properties of Thai sponge cake. The results of the current study showed that DSFP content (20-60% w/w) had significant effects on the appearance, color, odor, taste, texture, and overall liking of the Thai sponge cake (Table 4). The SSP content was the most important parameter affecting the sensory properties of the extrudate. Table 4 shows that increasing the DSFP from 20% to 40% resulted in increased liking scores for odor, taste, and overall liking. Thai sponge cake obtained from the highest DSFP had the minimum color scores. Because DSFP contains carotenoids, which have a yellow pigment. In addition, it also affects the texture quality; the DSFP had an effect on the puffiness of the Thai sponge cake. This is consistent with the research of Panichakornkul (2016) that supplementing DSFP in sandwich bread products; when considering physical quality, it was found that supplementing DSFP made the product with more dietary fiber affecting the structure of the bread, resulting the bread with more hardness, and firmness. It also showed that sandwich bread had a higher yellow color than the control sample. However, the liking score of odor, taste, texture, and overall liking

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had more than 6.0 score, especially for 20% w/w DSFP. Therefore, the levels of 20% w/w DSFP could be a desirable way to produce the healthy Thai sponge cake with appearance, odor, taste, texture, and an overall liking score of more than 6.00 which considered to be acceptable.

Table 4. Sensory evaluation of dried Sano flowers powder in Thai sponge cake.

Sensory characteristics	DSFP (%w/w)			
	Control	20	40	60
Appearance	7.12±0.93^{a}	6.32±0.79^{b}	6.40±0.85^{b}	6.78±1.03^{a}
Color	7.26±1.10^{a}	5.88±0.84^{c}	6.40±0.88^{b}	7.20±1.06^{a}
Odor	5.86±0.80^{c}	6.74±1.08^{a}	6.40±0.92^{ab}	6.12±1.00^{b}
Taste	5.76±0.68^{c}	6.66±1.23^{a}	6.24±0.84^{b}	6.10±0.83^{bc}
Texture	6.28±0.99^{b}	6.98±1.02^{a}	6.18±0.94^{b}	5.28±0.51^{c}
Overall liking	6.50±0.81^{b}	6.86±0.90^{a}	6.44±0.73^{b}	6.14±0.35^{c}

Mean ± SD with different lowercase superscripts in each low are significantly (p □ 0.05) different

3.4 Nutritional value of developed Thai sponge cake

The developed Thai sponge cake contained protein, fat, dietary fiber, ash, moisture and carbohydrates in 9.45, 5.23, 2.64, 1.16, 33.94 and 52.42 g/100 g respectively, and 319.10 µg/100 g β-carotene. The developed product supplemented with DSFP is a guideline to increase healthy nutrition especially dietary fiber, and β-carotene. The addition of DSFP in Thai sponge cake resulted in increased protein, fiber, and β-carotene, because the DSFP contains high dietary fiber and β-carotene. This is consistent with the research of Kijparkorn, Plaimast and Wangsoonoen (2010) reported that DSFP contained a lot of dietary fiber, and β-carotene, followed by lutein and β-cryptoxanthin. Khumkhom (2018) reported that the butter cookies with the DSFP had a high dietary fiber, and β-carotene when increasing DSFP. Therefore, it is possible to increase nutritional values by adding DSFP in healthy cake products.

Table 5. Nutritional values of developed Thai sponge cake.

Nutritional values	Developed Thai sponge cake 100 g
Moisture content (g)	33.94
Carbohydrates (g)	52.42
Protein (g)	9.45
Fat (g)	5.23
Dietary fiber (g)	2.64
Ash	1.16
β-carotene (µg)	319.10

4. Conclusion

In conclusion, the present study showed that the Sano flowers can be a material with agro-industrial potential with an important source of total carotenoids. Carotenoid has biological properties as antioxidant activity and antibacterial activity. Dried Sano flowers powder (DSFP) prepared and analyzed for total carotenoids. DSFP contained lutein, zeaxanthin and β-carotene. The effects of the addition with different levels of DSFP (20, 40 and 60 %w/w) of Thai sponge cake had been studied. An increase of the DSFP decreased the lightness (L*), redness (a*) value, springiness, and chewiness as well as yellowness (b*) value, hardness, cohesiveness, and gumminess. The sensory evaluation showed that all treatments obtained the overall liking score of 6.14-6.86. The study clearly showed that the levels of 20% w/w DSFP could be desirable to apply for producing the healthy Thai sponge cake. The developed Thai sponge cake contained protein, fat, dietary fiber, ash, moisture, and carbohydrates, indicating the DSFP is a guideline to increase healthy nutrition especially dietary fiber, and β-carotene. Therefore, the dried Sano flowers powder could enhance the quality of finished product and consumer satisfaction.

Acknowledgement

The authors would like to thank the Home Economics Program, Department of Applied Sciences, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok, Thailand for providing instruments.

Conflict of Interest

The authors would like to declare that there is no conflict of interest in this paper.

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Inhibitory Effect of Cadmium on the Growth of Mung Bean (*Vigna radiata* (L.) R. Wilczek) and the Removal by Chelating Agent

Chanya Phromchaloem[1*], Laphatsarapha Kumkaeo[1], Nattapornpash Insiripong[1], Laksamee Muensritharam[2]

[1]Department of Chemistry, Faculty of Science and Technology, Muban ChomBueng Rajabhat University, Chom Bueng, Ratchaburi 70150, Thailand

[2]Department of Food Processing, Faculty of Science and Technology, Muban ChomBueng Rajabhat University, Chom Bueng, Ratchaburi 70150, Thailand

*Corresponding author e-mail: chanyaphr@mcru.ac.th

Received: *2 March 2022* / **Revised**: *29 August 2022* / **Accepted**: *11 October 2022*

Abstract

This research studied the effect of cadmium (Cd) on the growth of mung bean (*Vigna radiata* (L.) R. Wilczek). The mung bean was cultured in a Hoagland solution containing different concentrations of Cd (0, 0.1, 0.3 and 0.5 mg/L) for 5 days. The result showed a significant decrease in the lengths of the roots and shoots of mung bean that was grown in cadmium solution. This effect was proportional to the concentrations of Cd. To assess cell death in the root of mung bean, Evan's blue staining technique was used in this study. The results showed that the concentrations of Evan's blue dye taken up by Cd-exposed mung beans at 0.1, 0.3, and 0.5 mg/L were 1.5612 ± 0.5417, 6.8641 ± 1.7447, and 8.0850 ± 2.6336 mg/L, respectively. A concentration-dependent increase of dead cells was found in the Cd-treated group, mostly at the root cap zone. With respect to this result, the level of dead cells that was stained with Evan's blue dye could be used as a biomarker to indicate cadmium contamination in water. Furthermore, the effects of chelating agents (EDTA) on cadmium removal were also studied. The results showed the possibility of using EDTA as a cadmium treatment agent and promoted plant growth in cadmium contamination areas.

Keywords: Cadmium toxicity, Mung bean, Evan's blue, Chelating agent

1. Introduction

Various technologies, particularly in the industrial and agricultural sectors, have been developed recently for convenience working. However, these developments, including mining, plating and agricultural industries, have had an impact on the environment. These industries have polluted the environment with toxic metals, which have been found in the soil, water, and air (Rafiq et al., 2014; Zhang & Reynolds, 2019). Some of these metals, like cadmium (Cd), are the hazardous substances in ecosystems that are most abundantly and omnipresently concerned with human health (Zhang & Reynolds, 2019). Due to their industrial applications, such as Ni-Cd batteries, color pigments, plastic stabilizers, smelters, and alloys, the amount of Cd output in the environment is dramatically increasing (Kubier, Wilkin, & Pichler, 2019). There have been reports of Cd contamination in soil and water in countries such as France, Belgium, China, South Korea, and Japan (Rizwan, Ali, Rehman, & Maqbool, 2019). The concentration of contamination varies by location, for example France has the highest Cd concentration in soil (16.7 mg/kg), followed by Belgium (7.61 mg/kg) and China (7.43 mg/kg). Cd concentrations in Taiwan were

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found to be 0.4 mg/kg however, after the establishment of the electroplating industry, it increased to 30 mg/kg (Rafiq et al., 2014). Pluemphuak, Mala, and Kumlung (2014) conducted a study on the contents of Cd that contaminated rice fields in Tak Province, Thailand, and found that Cd concentrations were greater than the standard limit (11.45-46.87 mg/kg).

Cd is absorbed and accumulated by plants, which can cause damage to the cell. This can be a physical and genetic influence on the plant (He, Yang, He, & Baligar, 2017; Parmar, Kumari, & Sharma, 2013). A number of studies have been conducted to investigate the effects of Cd on plants (Rizwan et al., 2019). Riceberry rice (*Oryza sativa*), for example, exhibited lower growth rate and pigmentation, as well as increased root cell toxicity, when exposed to high levels of Cd (Thaenghin, Pewnim, & Nakphayphan, 2017). A study of pea roots in Cd-contaminated wastewater treatment revealed that at Cd concentrations more than 30 mM, root growth changed significantly (Lima, Pereira, Figueira, Caldeira, & de Matos Caldeira, 2006). Furthermore, the influence of Cd on photosynthesis, nutrition, and growth of mung bean was studied, and the results showed that mung bean was resistant to low concentrations of Cd, and it was accumulated in roots, leaves, and stems (Wahid, Ghani, & Javed, 2008). Several studies have shown that Cd is hazardous to plants and other organisms, hence limiting consumption and minimizing Cd are crucial. Furthermore, Cd contamination should be detected early before it has a deleterious impact on ecosystems and public health.

Although measuring Cd concentration in water is an important method for determining the safety of water sources, it does not indicate the safe level of Cd concentration in aquatic organisms. A sensible approach for measuring the biological effect and assessing environmental quality is recently presented using cell or molecular biomarkers. Plants are sessile organisms susceptible to a range of stress factors such as Cd (Jaskulak & Grobelak, 2019). The ability of the cell to maintain changing external conditions is determined by the plant membrane, which can be used as a biomarker in the evaluation of cell damage or stress-induced death (Rizwan et al., 2019). Evan's blue staining technique has been established for the simultaneous monitoring of stress by cell death and membrane damage (Oprisko, Green, Beard, & Gates, 1990; Smith, Reider, & Fletcher, 1982; Vemanna et al., 2017). Evan's blue is a non-permitting, acidic exclusion dye that stains dead or damaged cells. The dye does not enter living cells with strong membranes. These stained cells can be characterized qualitatively by a light microscope. The blue dye can also be extracted from stained cells and quantified using a spectrophotometer. The amount of cells stained with blue dye under various conditions can be used as a measure of cellular stress by the correlation of dye in positively stained cells with the level of cell membrane damage (Vijayaraghavareddy, Adhinarayanreddy,Vemanna, Sreeman, & Makarla, 2017).

Mung bean (*Vigna radiata* L.) is a common legume crop throughout Asia and Thailand, and it is an important component of many cropping systems (Asim, Aslam, Hashmi, & Kisana, 2006). It is a food source and is cultivated for consumption and export. Thailand's exports to the Philippines, India and Singapore are approximately 1.26 million USD. According to the Organization for Economic Cooperation and Development (OECD) and the American Society for Testing Materials (ASTM), mung bean is an appropriate plant for toxicity experiments because it is sensitive to metal contamination and is also easy to grow and cultivate in a short period of time (Lee, Kwak, & An, 2012). As a reason, mung bean was chosen as the experiment plant for Cd toxicity in water in this study.

A chelating agent plays a key role in environmental adsorption of heavy metals. Ethylenediaminetetraacetic acid (EDTA) is a common chelating agent that is widely used in industry to sequester metal ions in aqueous solutions (Peraferrer, Martínez, Poch, & Villaescusa, 2012). EDTA is a hexadentate ligand that forms highly stable complexes with most transition metals, including Cd. It bonds these metals by forming a stable coordination complex with the donor atoms, two N atoms, and four O atoms of the unidentate carboxylate groups (Zheng, 2001). Because of its ability to bind metal ions, EDTA is frequently used

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for a range of applications, including reducing metal concentrations in contaminated environments. Studies on the adsorption of Cd and nickel metals in sunflowers by EDTA and HEDTA have been published. The study demonstrated the high efficacy of EDTA and HEDTA, although the plant species had limits (Chen et al., 2001). In addition, EDTA and citric acid were utilized for Cd water treatment with water hyacinth (*Eichornia speciosa*) (Kongmuang & Sampanpanish, 2010). Thus, the proposed study's aim was to investigate the effects of different Cd concentrations on mung bean growth and Cd toxicity using Evan's blue technique. This study also focused on the role of chelating agents (EDTA) in reducing the Cd effect.

2. Materials and Methods

2.1 Mung bean cultivation and treatment

Mung bean (*Vigna radiata* (L.) R. Wilczek) seeds were purchased from a supermarket in Chombueng, Ratchaburi, Thailand. Mung bean seeds germinated on wet tissue paper at room temperature and in the dark. After 24 hours, seedlings with root lengths of 1-2 mm were chosen and cultivated on diluted liquid Hoagland solution (Vijayaraghavareddy et al., 2017), containing $CdCl_2$ (Sigma-Aldrich Co., USA) concentrations of 0, 0.1, 0.3, and 0.5 mg/L. Cultivating the seedlings was accomplished by passing the roots through a plastic mesh disc (approximately 10 seedlings per 5 cm diameter disc). The discs were floating in 250 mL of solution that was kept under a light and dark cycle of 12:12 hours at room temperature for 5 days. The length of the root and shoot was measured every day of the experiment.

2.2 Quantification of membrane damage/ cell death using Evan's blue staining technique

According to Lehotai et al. (2011) Evan's blue staining was used to determine the toxicity of Cd on mung bean root. Evan's blue (Sigma-Aldrich Co., USA) solution at 0.1% (w/v) was being prepared. The mung bean root was cut into 1 cm lengths on ice and incubated in Evan's Blue solution for 15 min. As a positive control, the root was incubated in 1% Triton-X solution for 15 min. They were then rinsed thrice in distilled water or until unbound dye washes out from the root surface. Each mung bean root was photographed at 100x magnification using a light microscope (Olympus BX50). To quantify Evan's blue stain taken up due to membrane instability, the dye must be extracted from the roots using a mortar and pestle and homogenized in a destaining solution containing 1% Sodium Dodecyl Sulfate (SDS). Centrifuge the extract at 3,000 *x g* for 5 min at room temperature to elute the dye into the solution and to remove the debris. The absorbance of a blue supernatant was measured at 600 nm with a spectrophotometer using the destaining reagent as a blank. Concentration of Evan's blue can be estimated by referring to a standard curve. The amount of dye accumulated in positively stained cells correlates with the extent of cell membrane damage, hence the number of cells stained with Evan's blue dye can be used as a cellular death indicator. Samples from three independent experiments were measured with a pool of 10 plants.

2.3 Efficiency of Cd detoxification by chelating agents

In this study, EDTA was used as a chelator to evaluate the effects of chelating agents in reducing the Cd effect on Mung bean seedlings. There were five groups in the experiments. As a control group, the germinated mung bean was placed in a Hoagland solution. For the first experiment group, the germinated mung beans were placed in a Hoagland solution containing 0.5 mg/L EDTA. Secondly, the germinated mung beans were placed in a Hoagland solution containing 0.5 mg/L Cd. Thirdly, the germinated mung beans were placed in a Hoagland solution containing 0.5 mg/L EDTA and 0.5 mg/L Cd. Finally, the germinated mung beans were placed in a Hoagland solution containing 1 mg/L EDTA and 0.5 mg/L Cd. Root and shoot lengths were measured and recorded for 3 days in each experiment.

2.4 Statistical analysis

All the experiments were carried out in three replicates. The results are shown as mean ± SD.

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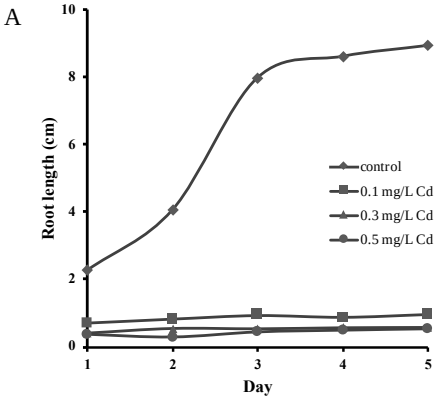
3. Results and Discussion

3.1 The effects of Cd on mung bean growth

According to Figure 1, the results showed that the growth of mung bean in the control and Cd treatment groups was clearly different, and the Cd concentration was inversely related to the growth. When compared with the control group, the length of the roots and shoots was inhibited in seedlings exposed to lower Cd concentrations and decreased significantly in seedlings exposed to higher Cd concentrations. This suggested that Cd had an effect on the root and shoot when it germinated, resulting in a decrease on mung bean growth. According to Figure 1A and 1B, Cd inhibited root growth since day one and caused brown stains on the roots. The shortening and browning of the roots exhibited in plants may suggest root cell damage caused by Cd. This can be explained by the fact that the root entered into direct contact and absorbed Cd before transferring it to the shoot. When Cd enters plant cells, Cd could inhibit protein synthesis, such as phosphoenol-pyruvate carboxylase (Shanmugaraj, Malla, & Ramalingam, 2019; Thaenghin et al., 2017), as well as bind to biological molecules, causing conformational and functional changes (Holubek et al., 2020). Cd, which is present in roots, shoots, and leaves, can accumulate in plant tissue and harm the plant throughout time (Wahid et al., 2008). According to this study, Cd may accumulate in a variety of mung bean parts, particularly the root. In this regard, the mung bean, like other leguminous plants, was sensitive to Cd (Aqeel et al., 2021; Geuns et al., 1997) and can be used to monitor the level of Cd in contaminated soil and water.



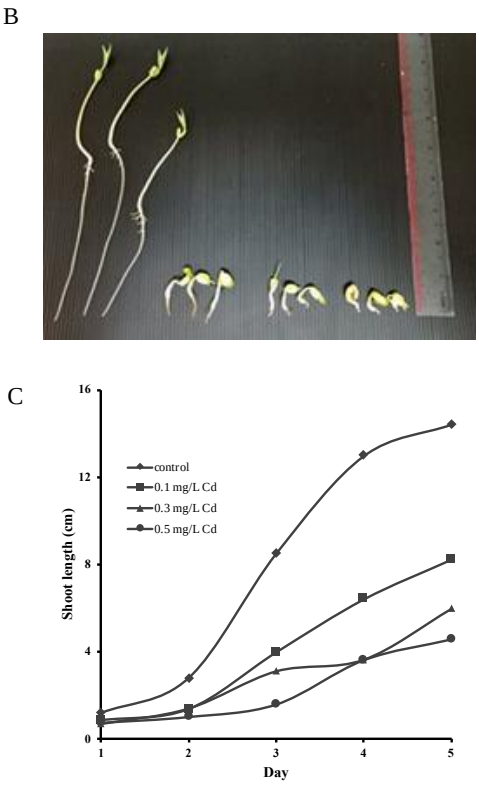


Figure 1. The growth of mung bean under various $CdCl_2$ concentrations for 5 days. A) Mung bean root growth decrease; B) Root and shoot morphology at day 5 ($CdCl_2$ concentrations of 0, 0.1, 0.3, and 0.5 mg/L from left to right); C) Mung bean shoot growth decrease.

3.2 Cell death and membrane integrity

Evan's blue staining technique was used to evaluate root cell damage caused by Cd exposure. The result showed that the roots of Cd-exposed mung bean seedlings uptake more blue dye than the control, suggesting that Cd caused membrane damage, permitting the dye to permeate through the cell. The higher concentration of Cd also resulted in a statistically significant increase in blue dye uptake, indicating an increase in the number of membrane damaged or dead cells. The Cd-exposed mung bean exhibited a concentration-dependent increase in dead cells, most of which were found in the root cap zone (Figure 2A). Plant cell membranes are semipermeable membranes that allow only certain substances to pass through cells. As a result, Evan's blue dye cannot pass through a living cell, but it can pass through death cells (Holubek et al., 2020). Furthermore, the concentration of Evan's blue dye in the root could well be conducted to assess Cd-induced root cell death (Thaenghin et al., 2017). The

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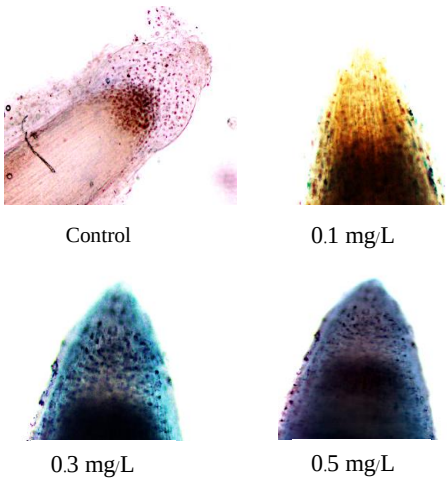
spectrophotometric method was used to assess root cell death by measuring the quantity of Evans' blue dye in the root. The results showed that the concentrations of Evan's blue dye taken up by Cd-exposed mung beans at 0.1, 0.3, and 0.5 mg/L were 1.5612 ± 0.5417, 6.8641 ± 1.7447, and 8.0850 ± 2.6336 mg/L, respectively (Figure 2B). According to the results of this study, the Cd-treated group absorbed more dyes than the control group. It also showed an increase in dye uptake that was relative to the higher concentration of the Cd-treated group, indicating a large increase in dead cells. This suggests that Cd causes cell death.

3.3 Efficiency of Cd detoxification by chelating agent

As a chelating agent for heavy metal adsorption, EDTA was employed in this study. According to Figure 3, the results showed that when chelators EDTA were mixed with Cd in a 1 to 1 ratio (0.5 mg/L Cd and 0.5 mg/L EDTA), there was a slight increase in growth of root and shoot of mung bean compared to the Cd treatment group on day 1 and a considerable increase on day 2 and day 3. When the ratio of EDTA and Cd was increased to 1 to 2 (0.5 mg/ L Cd and 1. 0 mg/ L EDTA) , there was a significant increase in root and shoot growth since day 1. This demonstrated that EDTA can minimize Cd toxicity and allow mung beans to grow normally in Cd- contaminated solution. According to the findings of several studies, EDTA could bind and remove heavy metals from Cd- contaminated conditions, which cause plant growth. Similarly, Bacaha et al. (2015) investigated the effect of Cd and EDTA treatment on plant growth (*Sorghum bicolor*).

A



Control 0.1 mg/L

0.3 mg/L 0.5 mg/L

B

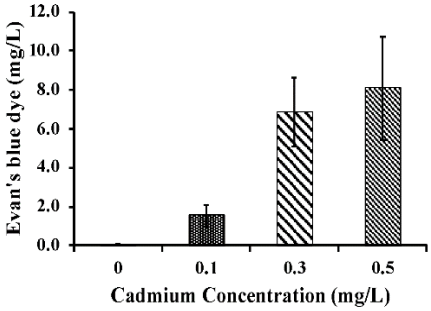


Figure 2. A) Microscopic detection of Evan's blue dye stain on mung bean root tips and B) the level of Evan's blue dye uptake in relation to the control by spectrophotometer measurements.

The results revealed that application of Cd and EDTA adversely affected shoot length, fresh weight and dry weight of *S. bicolor*. This implies that the hexadentate chelating agent EDTA could form a complex with Cd by bonding to it at six different sites. EDTA functions as a strong chelator to bind Cd, forming a Cd-EDTA complex which can reduce Cd accumulation and toxicity in plants. Additionally, Kongmuang and Sampanpanish (2010) investigated whether EDTA and citric acid may improve the performance of water hyacinth as a Cd adsorption agent in synthetic wastewater. The results showed that EDTA treatment could remove Cd more effectively than citric acid treatment.

The result of this study also found that EDTA alone had no effect on mung bean growth. Despite the fact that EDTA is a useful agent for Cd detoxification, it has a low harmful impact on both plants and the environment. Since EDTA degrades slowly and is a persistent organic pollutant, it can cause significant environmental problems. There have been some reports of concerns if we use it in high concentrations (Gonsior, Sorci, Zoellner, & Landenberger, 1997; Xie, 2009).

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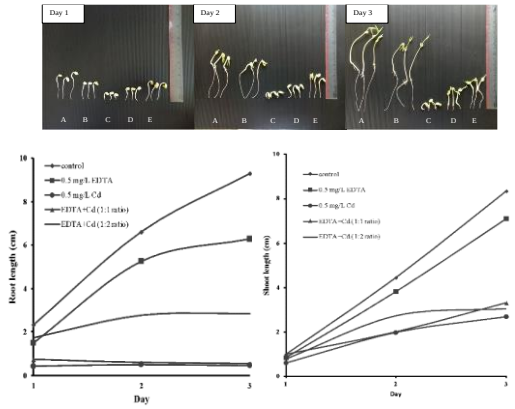

Figure 3. Morphology, root and shoot length of mung bean under EDTA and Cd treatments for 3 days, when mung bean germinated in A) distilled water, B) 0.5 mg/L EDTA, C) 0.5 mg/L Cd, D) 0.5 mg/L Cd and 0.5 mg/L EDTA (1:1 ratio), E) 0.5 mg/L Cd and 1.0 mg/L EDTA (1:2 ratio).

4. Conclusions

The results demonstrate clearly that Cd has an effect on mung bean root and shoot growth and is even harmful to plants in small concentrations. Following Cd exposure, the number of cell death stained with Evan's blue dye was found to be high in the mung bean root cap zone, indicating that Cd caused cell death. In a chelating agent application study, EDTA effectively detoxified Cd while also promoting root and shoot growth in mung beans. This is confirmed using chelators such as EDTA in the detoxification of heavy metals from the environment. According to Oviedo and Rodríguez (2003), EDTA has poor biodegradability, hence the study should use another chelator with greater biodegradability than EDTA. Mung bean plants grow well and much faster than other plants, and also show a reduction in root growth and cell death after Cd exposure. Instead of just employing costly and sophisticated methods to determine Cd, mung bean has the potential to be a sensitive tool or as a bioindicator for detecting Cd and monitoring Cd contamination in the environment. The most sensitive parameters to measure when using mung bean as a bioindicator for Cd exposure are root and shoot growth reduction, change in root tip brown color, and, most importantly, the quantity of root cell damage. However, additional study is needed to consider the possibility of Cd stress having an effect on pigment reduction, Cd accumulation, and other stress-induced changes in mung bean.

Acknowledgement

The support from the Department of Chemistry, Muban Chombueng Rajabhat University with their research facilities is highly appreciated.

Conflict of Interest

No conflict of interest.

ORCID

Author: Chanya Phromchaloem
https://orcid.org/0000-0003-2839-0007

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Game Method Based on Genre Game as Higher Educational Learning: Systematic Literature Review with VOSviewer

Soni Adiyono[1*], Sendi Novianto[2]

[1]Master Information System, Diponegoro University, Jl Imam Bardjo SH No 5, Pleburan, Semarang Selatan, Kota Semarang, Jawa Tengah 50241, Indonesia

[2]Informatics Engineering, Dian Nuswantoro University, Jl Imam Bonjol No 207, Pendrikan Kidul, Kec Semarang Tengah, Kota Semarang, Jawa Tengah 50131, Indonesia

*Corresponding author e-mail: sonisisu@gmail.com

Received: *2 March 2022* / **Revised**: *12 October* 2022 / **Accepted**: *1 November 2022*

Abstract

Game methods today have often been applied in several functions in the presentation in the realm of learning in multi-disciplines. The application of gaming methods is very varied to provide a different presentation for students to obtain a goal in the organization. In this case, the purpose of an organization is to increase the productivity and quality of a student. This paper discusses the methods and domains that are often used in the realm of education to achieve a goal. Systematic Literature Review is used in the application of research methods by finding selected articles with selected themes. In sorting the selected journals, inclusion and exclusion were also applied to obtain better article search results, and some 1,256 articles were found in the process, the application of filtering with exclusion and inclusion in this phase will result in 265 articles that fall into the category of candidate study discussion. The journal articles conclude that there are only 20 articles that can answer the questions in this paper. The results in this paper state that the Gaming Method in the realm of education is varied and the familiar game genre grouping used in the selected article is Simulation Game. The entire database in this research paper was obtained directly from the largest international journal alert database, namely Scopus. This is done by the database under the auspices of Elsevier as an organization or company publishing international scientific publications based in Amsterdam, the Netherlands since 1880.

Keywords: Game method, Student, Higher education, Systematic literature review

1. Introduction

The massive development of information technology is very rapid and sophisticated. This is evidenced by the proliferation of innovations that are emerging now, which indirectly greatly affects the realm of education and so does the learning system in it. Regarding the national education system which has been stated in law number 20 of 2003. Education works to develop capabilities and shape the character and civilization of a dignified nation in the context of the intellectual life of the nation. Based on the law, education aims to develop the potential of students to become human beings who believe and are devoted to God Almighty, have a noble character, are healthy, knowledgeable, capable, creative, independent and become democratic and responsible citizens (Schlickum, Hedman, & Felländer-Tsai, 2016).

The design that has been implemented by the UNESCO International Agency for Indonesia has 4 pillars of education, including: (Harjali, 2011; Schlickum et al., 2016)

1. Learn how to know
2. Learn how to do it
3. Learn how to be
4. Learn how to live together

The general space of a broad field of education, as well as its implementation, can be applied to all disciplines, and the methods used to achieve goals in

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a particular organization also vary. Educational games for health are designed to motivate individuals to improve their health through the implementation of changes and habits (Ferebee, 2010; Guo et al., 2021).

During the current COVID-19 pandemic, many teachers have developed effective learning plans for their students (Abouhashem et al., 2021). Referring to this, it is very necessary to have a new method to be able to provide motivation and a renewable treat, besides that there are quite a lot of methods applied in learning to obtain more optimal results in the goals of the organization. If we know more in this regard, it is in line with the research of Vusić et al. who adopted a game for the needs of cognitive interest and motivation to learn (Vusić, Bernik, & Geček, 2018; Winatha & Setiawan, 2020), it is also in the opinion of Stiller & Schworm who applies game-based learning in an educational context to achieve learning outcomes. educational goals for their students (Stiller & Schworm, 2019; Winatha & Setiawan, 2020).

The use of games as a learning tool requires pedagogical considerations for effective learning. Choosing the right game genre for learning is another important issue to consider when designing games for effective learning. Because the game genre gives its sensation to the game (Hidayat, 2018; Novayani, 2019).

This article is structured systematically to explain several things related to the game method applied in previous research and the grouping of game genres in each research according to journals that meet certain criteria. Therefore, this article was written to explain in more detail the related topics. which has been the focus of research on the application of developing methods and genres, and to add new information and links to readers related to the research carried out by the author.

2. Material and Methods

The method used in the preparation of this paper uses a systematic literature review approach. The database used in this paper was obtained from a reputable international journal from the publisher Scopus which was published in the 2015-2021 reference.

A systematic literature review also aims to search, find, and carry out a synthesis of journals or literature that has been systematically reviewed related to previous research, this method will go through several processes in it such as identification, evaluation, and interpretation of all available and relevant research that can answer questions related to topic areas or phenomena of interest than previous research (Ismail, Ramli, & Aziz, 2021; Jingga, Kosala, Ranti, & Supangkat, 2019). The stages in this systematic literature review method are as follows: (Jingga et al., 2019)

2.1 Literature review planning

2.1.1 Database selection

In this initial step, researchers tracked International Journals published by Scopus publishers with the domain of game methods applied in education with all scientific aspects in it.

2.1.2 Define keywords

After opening the official website portal for the Scopus publisher, the author selects the appropriate keywords in the search for related journals. Then the keywords that have been determined and used are

"game method" + "student" + "higher education"

2.1.3 Determine inclusion and exclusion criteria

In the previous step, when the keywords were determined and applied in the database search, the final results of the entire article were 1,256 documents. Subsequently, the following exclusion criteria were applied:

1. All Open Access
2. Vulnerable years of publication of the paper in the period 2016 – 2021 (last 5 years) Publication Stage = Final
3. Source Type = Journal

Language = English

TITLE-ABS

KEY (*game* AND *method*, AND *student*, AND *higher* AND *education*) AND (LIMIT-

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TO (PUBSTAGE , "*final*")) AND (LIMIT-
TO (OA , "*all*")) AND (LIMIT-
TO (PUBYEAR , *2021*) OR LIMIT-
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TO (PUBYEAR , *2018*) OR LIMIT-
TO (PUBYEAR , *2017*) OR LIMIT-
TO (PUBYEAR , *2016*)) AND (LIMIT-
TO (LANGUAGE , "*English*")) AND (LIMIT-
TO (SRCTYPE , "*j*"))

From the inclusion that has been applied at this stage, 265 documents have been produced that match the search criteria for that stage.

2.2 Featured article review

In the next stage, 265 documents have been obtained from the Scopus database which can be categorized as research that has been carried out by previous researchers. Researchers review each document to find appropriate journal documents regarding the game method applied in the scope of learning and cannot be separated from all disciplines in its application, then after the database on Scopus is displayed the initial screening of paper selection that meets the criteria, the screening results initially the author chose some 23 journals were selected to be reviewed in a more systematic detailed mapping of journals because of this journals contain the biggest keyword in VOSviewer, after doing some research about that 23 journals, we found that 3 journals did not meet the criteria for several reasons, including the following:

1. The publisher in the journal has discontinued status at Scopus.
2. There are publishers with newly discovered Scopus indexed status (E-ISSN:2664-9837) that have not been assigned a quartile.
3. Issuers with a not assigned quartile status.

So that by conducting initial screening and conducting exclusions at this stage, from the initial number of 23 journals selected to 20 journals that meet the criteria of authors who are included in the selected research category.

3. Results and Discussion

When going through several stages in this literature study, the author determines that there are 20 selected published journals from Scopus which will later be able to answer the Question Points (PP) set out in Table 1. Where the selected journals will discuss in more detail the method in the game itself, and also the classification in the game itself. genres applied in previous research.

Table 1. The arrangement of the parameters of the questions systematic literature review.

PP	Detail
PP1	What game method was applied in previous research?
PP2	What game genres have been applied in previous research?

The summary of selected journals will consist of selections that are formed into a detailed list in each selected journal, the variables include Author, Journal Title, Year of publication, Publisher (Publisher indexing Scopus), and Journal Ranking.

From the variable criteria that have been determined, the author in addition to looking for reputable International Journal data from Scopus as one of the reputable indexed publishers for scientific publications and a reputable scientific library database in the academic community around the world. The next step taken by the author is how to find out the ranking index for each of the selected journals, which have found as many as 20 selected journals?

Then, assisted by the portal page https://www.scimagojr.com/ (SJR Scimago) is a portal that can be accessed by the public which includes all journals and scientific indicators contained in the Scopus database. On this page, the author can apply indicators for each selected journal to obtain a ranking index for that journal which can be summarized in Table 2.

In this discussion, the keywords that have been determined will be searched for correlations with previous studies contained in the Scopus database as shown in Figure 1 below:

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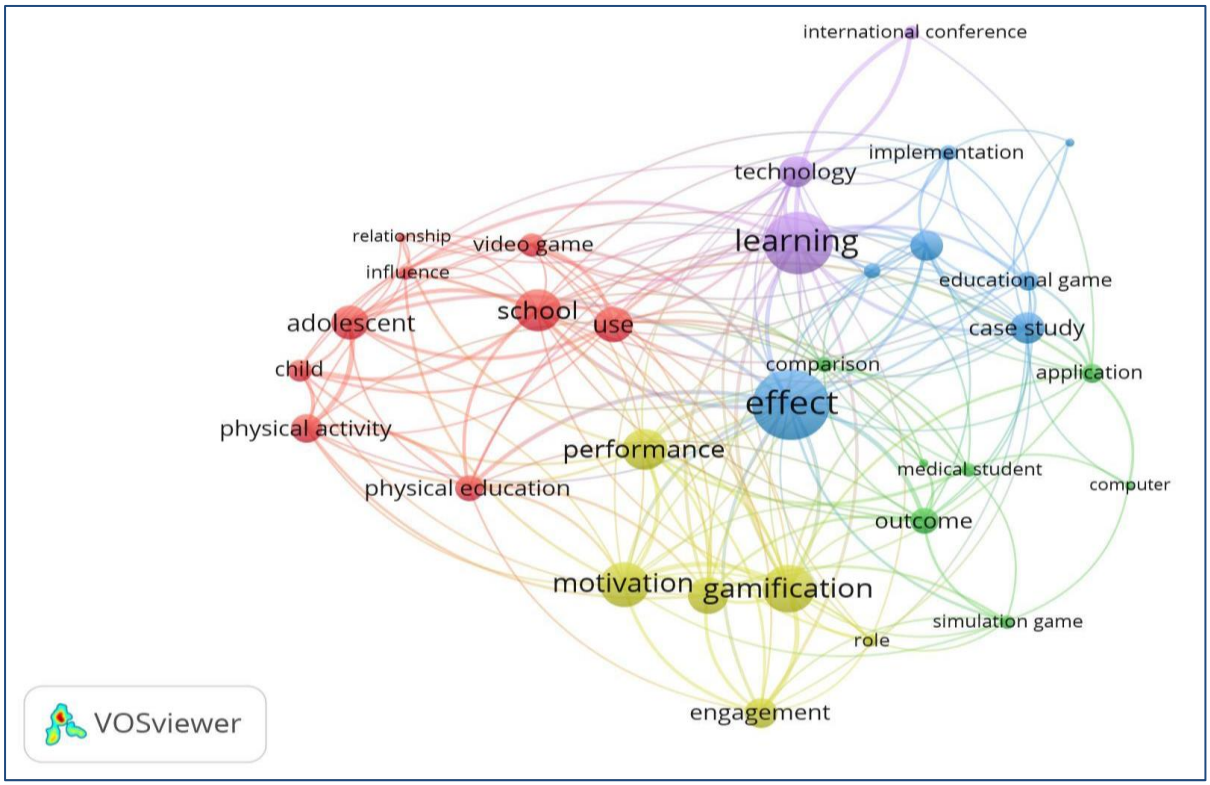

Figure 1. Visualization of the keyword network with search results in the Scopus database.

Table 2. Summary of selected Scopus journals.

Author	Title	Year	Publisher	Rank Journal
Jong-Long Guo, Hsiao-Pei Hsu, Tzu-Ming Lai, Mei-Ling Lin, Chih-Ming Chung and Chiu-Mieh Huang	Acceptability evaluation of the use of virtual reality games in smoking-prevention education for high school students: Prospective observational study	2021	Journal of Medical Internet Research	Q1
Azza Abouhashem, Rana Magdy Abdou, Jolly Bhadra, Malavika Santhosh, Zubair Ahmad and Noora Jabor Al-Thani	A distinctive method of online interactive learning in STEM education	2021	MDPI AG	Q1
Frederick K. Ho, Keith T. S. Tung, Rosa S. Wong, Ko Ling Chan, Wilfred H. S. Wong, Sai Yin Ho, Tai Hing Lam, Sheena Mirpuri, Benjamin Van Voorhees, King Wa Fu, Chun Bong Chow, Gilbert Chua, Winnie Tso, Fan Jiang, Michael Rich, Patrick Ip	An internet quiz game intervention for adolescent alcohol drinking: A clustered RCT	2021	American Academy of Pediatrics	Q1
Miroslav Popović, Klemo Vladimir and Marin Šilić	Application of social game context to teaching mutual exclusion	2018	Taylor and Francis Ltd	Q2
Tài Pham, François Beloncle, Lise Piquilloud, Stephan Ehrmann, Damien Roux, Armand Mekontso-Dessap and Guillaume Carteaux	Assessment of a massive open online course (MOOC) incorporating interactive simulation videos on residents' knowledge retention regarding mechanical ventilation	2021	BioMed Central Ltd	Q1

Friday Joseph Agbo, Solomon Sunday Oyelere, Jarkko Suhonen, and Teemu H. Laine	Co-design of mini games for learning computational thinking in an online environment	2021	Springer Nature Switzerland AG. Part of Springer Nature	Q1
Mary E. W. Dankbaar, Olivier Richters, Cor J. Kalkman, Gerrie Prins, Olle T. J. ten Cate, Jeroen J. G. van Merrienboer and Stephanie C. E. Schuit	Comparative effectiveness of a serious game and an e-module to support patient safety knowledge and awareness	2017	BioMed Central Ltd.	Q1
Meysam Siyah Mansoory, Mohammad Rasool Khazaei, Seyyed Mohsen Azizi and Elham Niromand	Comparison of the effectiveness of lecture instruction and virtual reality-based serious gaming instruction on the medical students' learning outcome about an approach to coma	2021	BioMed Central Ltd.	Q1
Audrey S. Pereira and Monika M. Wahi	Development and testing of a roleplaying gamification module to enhance deeper learning of case studies in an accelerated online management theory course	2021	The Online Learning Consortium	Q1
Heidi N. Eukel, Jeanne E. Frenzel and Dan Cernusca,	Educational gaming for pharmacy students – Design and evaluation of a diabetes-themed escape room	2017	American Association of Colleges of Pharmacy	Q1
Almudena Macías-Guillén, Raquel Montes Díez, Lucía Serrano-Luján and Oriol Borrás-Gené	Educational Hall Escape: Increasing motivation and raising emotions in higher education students	2021	MDPI AG	Q2
Viscione Ilaria and Romano Emanuela,	Elements and methods of organization, design and management of extracurricular sports activities	2019	Editura Universitatea din Pitesti	Q3
Guadalupe Molina-Torres, Irene Sandoval-Hernández, Carmen Ropero-Padilla, Miguel Rodriguez-Arrastia, Jesús Martínez-Cal and Manuel Gonzalez-Sanchez	Escape Room vs. traditional assessment in physiotherapy students' anxiety, stress and gaming experience: A comparative study	2021	MDPI Multidisciplinary Digital Publishing Institute	Q2
Juan Pimentel, Anne Cockcroft and Neil Andersson	Impact of game jam learning about cultural safety in Colombian medical education: A randomised controlled trial	2021	BioMed Central Ltd	Q1
Yanqiu Yu, Phoenix Kit-Han Mo, Jianxin Zhang, Jibin Li and Joseph Tak-Fai Lau	Impulsivity, self-control, interpersonal influences, and maladaptive cognitions as factors of internet gaming disorder among adolescents in China: Cross-sectional mediation study	2021	Journal of Medical Internet Research	Q1
María G. Gamero, Juan M. García-Ceberino, Sergio J. Ibáñez and Sebastián Feu	Influence of the pedagogical model and experience on the internal and external task load in school basketball	2021	MDPI Multidisciplinary Digital Publishing Institute	Q2
Dimitar Tomov, Daniela Tomova and Dimitar Atanasov	Tennis ball as a factor in the initial tennis training of junior high school (12-year old) students	2021	Editura Universitatea din Pitesti	Q3

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Hendra Saputra, Achmad Sofyan Hanif, Iman Sulaiman and Desy Tya Maya Ningrum	The effect of traditional games and drill with motor ability on skills (running, jumping, overhand throw and catching) at elementary school	2021	Horizon Research Publishing	Q4
María Consuelo Sáiz-Manzanares, Caroline Françoise Martin, Laura Alonso-Martínez and Leandro S. Almeida	The usefulness of digital game-based learning in nursing and occupational therapy degrees: A comparative study at the University of Burgos	2021	International Journal of Environmental Research and Public Health	Q1
Marcus Schlickum, Leif Hedman and Li Felländer-Tsai	Visual-spatial ability is more important than motivation for novices in surgical simulator training: A preliminary study	2016	International journal of Medical Education	Q2

From the summary of the 20 selected journals above, it was found for the index of journals with a Q1 rating of 12, a Q2 ranking of 5, a Q3 rating of 2 and in Q4 there was 1 selected journal. Thus, to answer questions about this research and provide a more detailed understanding of the selected journals, the researchers found a game method that was applied to previous research and the game genres in this study.

A genre can be defined as the characteristics of a game, where these characteristics are characterized by several similarities, including content, style, and gameplay (Novayani, 2019) or more familiarly, this genre itself can be interpreted as grouping games or games and in it, there are no standard rules that bind to the grouping itself (Novayani, 2019). For details on game methods and game genre groupings in the selected journals, see Table 3.

Table 3. Details of selected journal game methods & genres.

References	Journals Detail		Gaming Method	Game Genre
	Title	Publisher		
Guo et al. (2021)	Acceptability evaluation of the use of virtual reality games in smoking-prevention education for high school students: Prospective observational study	Journal of Medical Internet Research	Game VR (Virtual Reality)	RPG (Role-Playing Game)
Abouhashem et al. (2021)	A distinctive method of online interactive learning in STEM education	MDPI AG	Online Interactive Learning	Simulation Game
Ho et al. (2021)	An internet quiz game intervention for adolescent alcohol drinking: A clustered RCT	American Academy of Pediatrics	Internet Quiz Game	Puzzle Game
Popović, Vladimir and Šilić (2018)	Application of social game context to teaching mutual exclusion	Taylor and Francis Ltd	Social Game Learning	RPG (Role-Playing Game)
Pham et al. (2021)	Assessment of a massive open online course (MOOC) incorporating interactive simulation videos on residents' knowledge retention regarding mechanical ventilation	BioMed Central Ltd	Interactive Simulation	Simulation Game
Agbo, Oyelere, Suhonen and Laine (2021)	Co-design of mini games for learning computational thinking in an online environment	Springer Nature Switzerland AG. Part of Springer Nature	Prototype Mini Game Online	Strategy Game

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Dankbaar et al. (2017)	Comparative effectiveness of a serious game and an e-module to support patient safety knowledge and awareness	BioMed Central Ltd.	Serious Game	Puzzle Game
Mansoory, Khazaei, Azizi and Niromand (2021)	Comparison of the effectiveness of lecture instruction and virtual reality-based serious gaming instruction on the medical students' learning outcome about an approach to coma	BioMed Central Ltd.	Virtual Reality (VR)-Based Serious Gaming	RPG (Role-Playing Game)
Pereira and Wahi (2021)	Development and testing of a roleplaying gamification module to enhance deeper learning of case studies in an accelerated online management theory course	The Online Learning Consortium	Game Based-Learning	Simulation Game
Eukel, Frenzel and Cernusca (2017)	Educational gaming for pharmacy students – Design and evaluation of a diabetes-themed escape room	American Association of Colleges of Pharmacy	Innovative educational game	Puzzle Game
Macías-Guillén, Díez, Serrano-Luján and Borrás-Gené (2021)	Educational Hall Escape: Increasing motivation and raising emotions in higher education students	MDPI AG	Game Based-Learning	Simulation Game
Ilaria and Emanuela (2019)	Elements and methods of organization, design and management of extracurricular sports activities	Editura Universitatea din Pitesti	Teaching games for understanding	Action Game
Molina-Torres et al. (2021)	Escape Room vs. traditional assessment in physiotherapy students' anxiety, stress and gaming experience: A comparative study	MDPI Multidisciplinary Digital Publishing Institute	Gaming Experience	RPG (Role-Playing Game)
Pimentel, Cockcroft and Andersson (2021)	Impact of game jam learning about cultural safety in Colombian medical education: a randomised controlled trial	BioMed Central Ltd	Game Jam Learning	Simulation Game
Yu, Mo, Zhang, Li and Lau (2021)	Impulsivity, self-control, interpersonal influences, and maladaptive cognitions as factors of internet gaming disorder among adolescents in China: Cross-sectional mediation study	Journal of Medical Internet Research	Internet Gaming	Simulation Game
Gamero, García-Ceberino, Ibáñez and Feu (2021)	Influence of the pedagogical model and experience on the internal and external task load in school basketball	MDPI Multidisciplinary Digital Publishing Institute	Tactical Games Approach (TGA)	Strategy Game
Tomov, Tomova and Atanasov (2021)	Tennis ball as a factor in the initial tennis training of junior high school (12-year old) students	Editura Universitatea din Pitesti	Sports Game	Action Game

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Saputra, Hanif, Sulaiman and Ningrum (2021)	The effect of traditional games and drill with motor ability on skills (running, jumping, overhand throw and catching) at elementary school	Horizon Research Publishing	Traditional Games	RPG (Role-Playing Game)
Sáiz-Manzanares, Martin, Alonso-Martínez and Almeida (2021)	The usefulness of digital game-based learning in nursing and occupational therapy degrees: A comparative study at the University of Burgos	International Journal of Environmental Research and Public Health	Game Based-Learning	Simulation Game
Schlickum et al. (2016)	Visual-spatial ability is more important than motivation for novices in surgical simulator training: A preliminary study	International Journal of Medical Education	laparoscopic surgical simulator	Simulation Game

Table 3 shows that for game genres that have been grouped according to the genre applied in the gaming method from previous research, there are 5 different classification groups (Novayani, 2019), which in this genre are also reviewed from the aspect of the gaming method used in previous studies.

The results of the analysis in Table 3 can be visualized in Table 4 and Figure 2, where after being integrated into the visualization it will appear that in the 20 selected journals the methods used to achieve research objectives are very varied, but in grouping this genre is dominant. entered into the Simulation Game genre, which proved to be 8 out of 20 or 0.40 (Abouhashem et al., 2021; Ismail et al., 2021; Jingga et al., 2019) per cent, then the RPG (Role-Playing Game) genre dominated in second place with a total of 5 or 0.25 per cent, while the Puzzle Game genre contained 3 out of 20 selected journals which reached 0.15 per cent, while in the Strategy and Action Game genres, the number was relatively the same, namely 2 of the 20 selected journals which achieved 0.10 per cent.

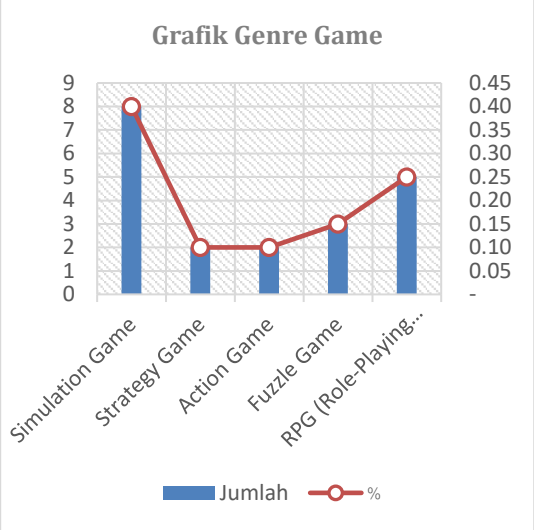

Figure 2. Graph of selected journal game genre grouping results.

Table 4. Detailed results of selected journals.

Genre	Total	%
Simulation Game	8	0.40
Strategy Game	2	0.10
Action Game	2	0.10
Puzzle Game	3	0.15
RPG (Role-Playing Game)	5	0.25

4. Conclusions

The results of the search with keywords that have been carried out by the author are a total of

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1,256 documents in the Scopus database, then after the inclusion and exclusion criteria, it decreases significantly and results in 265 documents.

From these results, a more detailed screening was carried out and found 20 articles were selected for research discussions. Then from the results of the selection of the 20 journals, they analyzed the game method applied in each previous research, and there are variations in the application of the game method. Moreover, in grouping the genre, Simulation games dominate more in a study based on the Scopus database in the last 5 years. This can be because the Simulation game genre is easier to use. After all, it provides knowledge to simulate things from different dimensions and scales.

From the research author assumes that the application of the game method in the realm of learning will provide a different stimulus and achievement, this is because students will enjoy and feel they are not experiencing the learning process which is actually in the packaging of certain game methods will realize goals rather than what will be individual goals as well as the organization, namely achieving superior (Stiller & Schworm, 2019).

ORCID

Author
Soni Adiyono, S.Kom
Education and Qualification
Bachelor (Computer Science) Education,
Dian Nuswantoro University, Indonesia
Student at master information system,
Diponegoro University, Indonesia

Dr Sendi Novianto, S.Kom MT
Education and Qualification
Bachelor (Computer Science),
Dian Nuswantoro University, Indonesia
Master of Game Technology, Institut Teknologi Sepuluh November, Indoensia
Doctor of Philosophy – PhD Control Theory & Control Engineering, South China University of Technology, China
https://orcid.org/0000-0002-3462-7958
ResearcherID: H-3920-2016
Scopus Author ID: 56121388400
Lecture at Informatics engineering,
Dian Nuswantoro University, Indonesia

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Available online at https://li02.tci-thaijo.org/index.php/ssstj

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A Systematic Review on Knowledge, Attitude and Practices (KAP) of Food Safety among School Children: A Global Perspective

Piyumi Chathurangi Wanniarachchi*, Piumi De Abrew Abeysundara

Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, 10250, Sri Lanka

***Corresponding author e-mail: piyumiwanniarachchi@sci.sjp.ac.lk**

Received: *18 April 2022* / **Revised**: *29 August 2022* /**Accepted**: *6 December 2022*

Abstract

Worldwide there are lack of studies demonstrating the knowledge, attitude, and practices (KAP) about food safety and hygiene, among school children. Thus, this systematic review is an attempt to summarize the findings in previous literature about food safety knowledge, attitude, and practices among school children, in a specific time period, to get an overview of the topic. This review was performed according to PRISMA protocol. Three main international databases (Google Scholar, PubMed and Scopus) were used for the search of the articles, and only the journal articles published from 2010 to 2020 were considered. Of the 102 articles referred, only 10 original research studies were selected based on the criteria for inclusion and exclusion. Only 2 out of 10 studies pointed out significant correlations between the school children's food safety knowledge, practices and attitudes. In addition, 1 out of 10 studies demonstrated significant associations between common demographics (academic performance of the children, the type of school, the area of residence, the habit of smoking and alcohol consumption and the educational background of the parents) and food safety knowledge among children. Furthermore, 8 out of 10 studies reported students to possess a poor or average knowledge of food safety while the rest of the studies showed students to possess good knowledge regarding food safety. Only 3 out of 10 studies revealed students to be engaged in safe food handling practices. It can be concluded that the assessment of food safety knowledge, attitude and practice as the most productive initiative to comprehend food safety-related insights among school children. Furthermore, this review highlights the fundamental requirement to improve children's food safety knowledge and practices through proper education.

Keywords: Food safety, Knowledge, Attitude, Practices

1. Introduction

Each and every year a considerable number of foodborne disease outbreaks and an increased number of victims are reported primarily due to the usage of contaminated food or water. Statistics from the World Health Organization reported that nearly two million people are killed annually due to diarrheal diseases and a clear majority of them represent children in developing and under-developed countries (World Health Organization [WHO], 2020). In the past decades, there has been an increase in the outbreak of many foodborne diseases worldwide due to weak and inadequate food safety regulations and the absence of proper education, training and skills among people who are involved in food preparation (Sharma, Srivastava, Banerjee, & Shaili, 2018).

Often, food becomes unhealthy for consumption once it is exposed to biological, physical or chemical hazards (Fung, Wang, & Menon, 2018; Kamboj, Gupta, Bandral, Gandotra, & Anjum, 2020; Kwol, Eluwole, Avci, & Lasisi, 2020). Every food has a vulnerability to getting contaminated at any phase during food handling. Incorrect time and temperature control associated with food during the supply chain, unsafe food processing/handling, inadequate cooking, cross contamination and improper personal hygiene practices were often described as causes for foodborne disease outbreaks (Ali & Immanuel 2017; Sani & Siow, 2014; Webb & Morancie, 2015). Severe cases of hospitalization and death that occurred as a result of food contamination highlight the danger of foodborne diseases. Furthermore, these

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extreme cases suggest that the individuals must possess adequate food safety knowledge, from childhood in order to minimize the risks associated with foodborne disease outbreaks.

School-aged children are one of the most important and sensitive social categories, who are often susceptible to food-related risks. According to some recent research, if children possess adequate food safety knowledge and proper hygienic practices, many foodborne illnesses can be prevented or minimized (Wanniarachchi, Abeysundara, & Peiris, 2021a, 2021b). Thus, having an overview of the food safety KAP of school children is important to researchers in the fields of public health and food safety.

A limited number of studies have been published about food safety KAP of school children (Wang, Wang, & Cai, 2013). Therefore, the objective of the current study is to summarize the selected findings from the previous literature about food safety KAP among school children around the world, from 2010-2020 to get an overview of the topic. Based on the gathered information, this work will assist the early career researchers in the field of public health and food safety who are interested in studying about food safety aspects of different communities within the population.

2. Materials and Methods

The review was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol (Moher, Liberati, Tetzlaff, & Altman, 2009). The present study is a systematic literature review. The articles were searched in three international databases, namely Google Scholar, PubMed and Scopus. The search was restricted to journal articles from peer-reviewed, open access, indexed journals and the language of interest was English. Articles published from 2010 to 2020 were considered for the review process. Initially, the keywords; (food safety, knowledge, attitude, and practices) were used for searching relevant articles. Through the initial search of articles, the authors found 102 articles with at least one of the above keywords including review papers and original research papers.

The review papers Included in the above set of 102 articles were disregarded while keeping the original research papers only. Further, exclusion was done to get rid of the papers whose target populations are different from school children even though they described KAP regarding food safety.

Next, as the inclusion criteria, any original research paper's title that contained at least two of the keywords (knowledge, attitude, practices) accompanied by the keyword "food safety", was encompassed in the review (Table 1). Finally, the selected articles were read completely to guarantee their suitability for our purpose.

Of the 102 articles referred, only 10 papers were selected based on the criteria for inclusion and exclusion. The final search of the articles was conducted in May 2021.

3. Results and Discussion

Table 1 depicts the summary of 10 selected previous studies about food safety KAP among school children.

3.1 Questionnaire designing and data analysis

In almost all studies, questionnaires have been created based on similar, previously validated questionnaires, the country's health and safety regulations and preventive measures and other safety guidelines introduced by the World Health Organization to ensure food safety. Of the 10 studies under consideration, 4 have paid attention completely to the three words "Knowledge, attitude and practices" (Cheng, Zhang, Ma, & Zhan, 2017; Majowicz et al., 2015; Moghaddam, Hassanzadazar, Vakili, Jafari, & Aminzare, 2020; Ovca, Jevšnik, & Raspor, 2014) while the rest of the papers paid attention at least on two of the above terms. In all the research works of interest here, data collection has been directed through questionnaires. The software used in the formal analysis and the particular tests performed to draw meaningful insights and conclusions in those 10 studies, were carefully summarized by the authors of the present study. Different software such as Stata/SE (14.0, 14.1), Epi Data Entry (Chinese version 3.1), SPSS (15.0 /16.0/17.0/ 20.0/ 22.0), SAS 9.4, Epi Info 7 software and Microsoft Excel 2016 were used. Depending on the normality or non-normality of data being handled different tests such as (Descriptive statistics, independent sample t-tests, Paired t-test, Duncan's pairwise comparison, Pearson's chi-square test, Fisher's exact test, Multivariable Poisson and logistic regression analysis, Multiple linear regressions, Cronbach's alpha, Analysis of variance, Cross tabulations, Spearman's correlation

coefficient, Pearson correlation coefficient, Kruskal-Wallis test, Wilcoxon rank-sum, Multiple comparisons- SNK-Q test) were used by studies, as relevant to examine the relationships among and between the variables, to build statistical models and draw meaningful insights from the data (Table 1).

3.2 Characteristics of the participants

Among 10 studies under review, 2 studies have been carried out in Canada (Majowicz et al., 2015; Majowicz et al., 2017), while the other studies have been carried out in countries like China, the USA, Ghana, United Arab Emirates, Jordan, Slovenia, Iran and Malaysia. 2 out of 10 studies have chosen school children in the age group 10 to 12 years as their target population (Ovca et al., 2014; Tutu, Hushie, Asante, & Egyakwa-Amusah, 2020), while 4 studies have paid interest on children between 13-19 years of age (Cheng et al., 2017; Majowicz et al., 2015; Moghaddam et al., 2020; Norazmir et al., 2012). The rest of the studies haven't specifically mentioned the age group of the participants. About 4 out of 10 studies have employed samples greater than 1000 participants (Cheng et al., 2017; Majowicz et al., 2015, Ovca et al., 2014; Tutu et al., 2020) while others have used sample sizes of less than 1000 participants.

3.3 Food safety KAP vs demographics of school children

Altogether, 7 out of 10 studies under consideration contained a separate section in the questionnaires to provide socio-demographic characteristics of the participants (Cheng et al., 2017; Majowicz et al., 2015; Majowicz et al., 2017; Moghaddam et al., 2020; Norazmir et al., 2012; Osaili, Obeidat, Jamous, & Bawadi, 2011; Tutu et al., 2020). Food safety knowledge, as well as their practices within a population, differ by subpopulations based on demographic and geographic characteristics (Moreb, Priyadarshini, & Jaiswal, 2017). Demographic details such as gender, age, the number of siblings, the school category, grade, height and weight of the child, ethnicity, the average monthly income, the residential area, the parents' occupation and the parents' education background, etc. Have been studied for their effects on the KAP of school children (Table 1). Only 1 out of 10 studies reported, significant associations between common demographics such as academic performance of students, the type of school, the area of residence, habit of smoking and alcohol consumption and education background of parents with food safety KAP of students.

3.4 Food safety attitude of school children

A good attitude is one of the key factors in the conversion of knowledge into proper food handling practices (Da Cunha, Stedefeldt, & De Rosso, 2014). Altogether, the papers under review here contained 3 to 17 attitude-based questions. About 6 out of 10 studies have directly evaluated the school children's attitude on food safety through the questionnaire (Byrd□Bredbenner, Abbot, & Quick, 2010; Cheng et al., 2017; Majowicz et al., 2015; Majowicz et al., 2017; Moghaddam et al., 2020; Ovca et al., 2014). Of the 6 studies which addressed students' attitudes toward food safety, only one study reported students to possess a favourable attitude toward food safety. Furthermore, these studies revealed, the need for school children to receive, further education on food safety (Byrd-Bredbenner et al., 2010; Cheng et al., 2017; Majowicz et al., 2015; Majowicz et al., 2017; Osaili et al., 2011; Ovca et al., 2014).

Byrd-Bredbenner et al. (2010) revealed that many middle schoolers tend to believe that preparing food alone can cause food poisoning, owing to the reasons like lack of previous food preparation experiences, poor knowledge or due to poor food handling practices. Therefore, in such cases upgrading their knowledge as well as confidence regarding food safety-related aspects is a must to ensure food safety. Researchers pointed out, the need for a synergetic endeavor among different parties such as school children, their parents, food safety professionals and educators to improve food safety awareness among them (Getty, 2014; Horikawa, Akamatsu, Horiguchi, & Marui, 2013).

The above results are further backed up by a recent study carried out in Sri Lanka, where the majority (92.4%) of the school children were self-assured that their current level of knowledge on food safety could be further improved by the provision of proper education. The same study revealed parents, teachers and doctors as pioneer food safety educators in the Sri Lankan context (Wanniarachchi, Abeysundara, & Peiris, 2021c).

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Table 1. Summary of the selected studies carried out about food safety knowledge, attitudes and practices of school children, worldwide from 2010 – 2020.

Authors, year and country of the study	Attributes of the participants of the study	Main objective/objectives	Nature of the questions included in the questionnaire	Primary findings
Majowicz et al. (2015) Canada	(n = 2,860) high school students of four Ontario colleges Age- 13 to 18 years	• To evaluate food safety KAP of high school students in Ontario	1. Demographic details of participants 2. Questions about food safety knowledge – 4 questions 3. Questions to check attitude – 4 questions 4. Questions regarding self-reported food safety practices -7	• Overall food safety knowledge of the participants was low. • Majority of the participants (72.7%) were confident that they could cook safe, meals for both their families and themselves. • More often, students were found to follow good hygienic practices while handling food. • Necessity of further education on food safety was evident.
Cheng, Zhang, Ma and Zhan (2017) China	(n = 4,220) Students in 3 districts of Beijing. Age- 13 to 18 years	• To understand and build meaningful insights on the food safety KAP among students in Beijing	1. Demographic details 2. Questions about food safety knowledge – 7 questions 3. Questions to check attitude – 3 questions 4. Questions regarding self-reported food safety practices -3 questions	• Overall food safety knowledge of the study group was good. • Significant correlations were shown between food safety knowledge scores and demographic data such as residential area, type of school, the habit of smoking and alcohol consumption, academic performance in the class and the parents' education background. • The study highlighted the need for providing further food safety education to children for a safer tomorrow.
Norazmir et al.	(n = 339)	• To assess knowledge and practice regarding food safety among	1. Demographic details	• Overall food safety knowledge and practice of the participants were good.

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(2012) Malaysia	Secondary School children in Johor Bahru, Malaysia Age 16 to 19 years	secondary schoolers in Johor Bahru, Malaysia ● To demonstrate the relationship between food safety knowledge and practices with gender. ● To determine the correlation between food safety knowledge and practice scores.	2. Questions about food safety knowledge- 10 items 3. Questions about food safety practices -10 items	● No significant differences in food safety knowledge and practice were observed between genders. ▪ Weak and positive correlations between food safety knowledge and practice were noted.
Ovca, Jevšnik and Raspor (2014) Slovenia	(n=1272) 26 primary schools in the district Ljubljana and nearby in Slovenia Age- 10 to 12 years	● To gain meaningful insights into the experiences of children with food preparation ● To understand food safety risks and minimize getting exposed to them ● To evaluate KAP on food safety among students in Slovenia.	1. Students' experiences with food preparation and food handling -5 questions 2. Students' personal understanding of food safety risks - 6 statements 3. Third section -Food safety knowledge - 18 questions 4. Statements regarding self-reported food safety practices – 15 statements	● Children were found to engage in food preparation with limited experience. ● Poor knowledge on the impact of proper temperature control to assure microbial food safety was evident. ● Results demonstrated the necessity for students to receive further education and guidance regarding food safety.
Tutu, Hushie, Asante and Egyakwa-Amusah (2020) Ghana	(n=1343) School children in the Ga West (Upper primary and Junior high school students)	● Primarily to assess food safety knowledge and practices of the participants of the study.	1. Demographic details 2. Food safety knowledge – 9 questions	● Poor food safety knowledge was depicted among students. ● Majority of the students engaged in appropriate food safety practices. Eg. Hand washing

	Age- 10 to 12 years		3. Food safety practice – 9 questions	• Positive correlations between food safety knowledge and practices were shown. • More than half of the participants insisted to look for the cleanliness and the neatness of the surroundings first, followed by the vendor's attributes before they try food from outside.
Moghaddam, Hassanzadazar, Vakili, Jafari and Aminzare (2020) Iran	(n=640) High school children in in Khorramdarreh, Zanjan, Iran Age- 14 to 19 years	• To assess food safety KAP among high school children in Khorramdarreh, Zanjan, Iran	1. Demographic information 2. Questions to test food safety attitude-15 items 3. Questions to test safety knowledge-24 items 1. Questions to test food safety practices-10 items	• Overall, students depicted moderate knowledge of food safety. • Similarly, favourable level of food safety attitude and practice were observed among children. • Students depicted poor knowledge of the effects of temperature on food (35%) • Training regarding food safety has been recommended by the authors to be given to children as well as parents to upgrade their knowledge on food safety.
Majowicz et al. (2017) Canada	(n = 119) high school children in Ontario, Canada	• To assess food safety knowledge and attitude of high school students. • To determine whether food safety knowledge and attitude have upgraded after taking part in a training program on proper food handling.	1. Food safety knowledge questions – 7 items 2. Food safety attitude questions – 17 items 3. Demographic details and food handling experience questions- 8 items	• Poor food safety knowledge and attitude among high school students was reported. • Authors recommend in-class delivery of existing programs, as effective in order to enhance students' knowledge of food safety. • The study revealed that the assessment that 3tilize observational data to demonstrate the impact of food safety education on students' genuine food handling behavior as important.
Osaili, Obeidat, Jamous and Bawadi	(n=867)	• To investigate the association between food safety knowledge and socio-demographic and academic	1. Demographic and academic characteristics of students	• A satisfactory level of knowledge on prevention of cross-contamination and disinfection procedures was reported.

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(2011) Jordan	school children (only females)living at dorms	variables among college female students in the north of Jordan.	2. Questions to assess students' knowledge of food safety	● The participants reported poor knowledge food foodborne pathogens. ● Need to provide food safety educational interventions covering major food safety concept was observed.
Abushelaibi, Jobe, Al Dhanhani, Al Mansoori and Al Shamsi (2016) United Arab Emirates	(n=147) Students of eight public schools in the city of Al Ain, United Arab Emirates	● To assess food safety knowledge and hygienic practices among students in selected schools in Al Ain city UAE.	1. Questions to demonstrate the behavior of participants about food safety aspects. 2. Questions to assess students' knowledge and practices about food safety 3. Questions to assess participants' awareness about food contamination and hand hygiene.	● About 75% of the schools were found not to clean their refrigerators (where the food is stored) enough periodically. ● Results demonstrated that the majority of students did not practice proper hand washing after using the toilet. ● Microorganisms like *Escherichia coli* and *Staphylococcus aureus* were found in hands of school children, food handlers and teachers.
Byrd□Bredbenner, Abbot and Quick (2010) The USA	Middle school children in 5 major geographic regions of New Mexico, in the USA Parents of the above-mentioned middle schoolers (participated in baseline parent focus groups) Professionals on food safety	● To explore the responsibilities of school children during food processing and handling ● To explore the hindrances to be faced during the practicing of safe food handling and develop strategies to overcome them.	4. A qualitative research design involving mainly 3 focus groups	● Youth pointed out that food can make them sick, and explained reasons such as substance/microorganisms entering into food, and inappropriate cooking as causes for foodborne illnesses. ● Almost all the participants were keen on learning about food safety. ● Parents showed high enticement over students to learn further about food safety. ● Parents suggest food safety education be given to students at schools and in domestic settings.

- Parents suppose that their children have average levels of food safety knowledge, but doubted them to practice safe food handling behaviors while unsupervised.

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3.5 Food safety knowledge of school children

In almost all studies listed in Table 1, the food safety knowledge of school children has been evaluated. In order to assess food safety knowledge, areas such as microbial contamination of kitchen countertops and utensils, prevention of cross-contamination, temperature regulation to ensure microbial safety of food, common food sources of food borne-diseases, the application of proper heat treatment, monitoring food before consumption, basic disinfection procedures and knowledge about basic food safety principles and personal hygiene were tested. Of the 10 studies considered, 8 studies reported students to have poor or average food safety knowledge. Only 2 out of 10 studies demonstrated students to have good knowledge on food safety (Cheng et al., 2017; Norazmir et al., 2012). School children who participated in the studies reported poor knowledge with regard to proper temperature control on microbial growth and survival, the shelf life of food and common foodborne pathogens (Moghaddam et al., 2020; Osaili, et al., 2011; Ovca et al., 2014). Meantime, students were knowledgeable about maintaining personal hygiene while preparing food (e.g., basic hand washing), disinfection procedures, averting cross-contamination during food preparation and handling) (Abushelaibi et al., 2016; Osaili et al., 2011; Ovca et al., 2014).

Since the majority of studies did not show, adequate food safety knowledge among students, it creates a need to improve awareness among them regarding basic principles of safe food handling. School children's knowledge with regard to proper temperature control on microbial growth and survival and common foodborne pathogens needs to be improved. For this, the authors of the present study suggest, that educational programs and training programs be conducted in school settings where children are more attentive to learning with peers. And also, the responsible authorities regarding school children's education can pay attention to including the relevant content about food safety in their school curricula to make them more familiar with the content (Ovca et al., 2014).

3.6 Food safety practices of school children

Only 3 out of 10 studies reported students to be engaged in appropriate and safe food handling practices (Majowicz et al., 2015; Norazmir et al., 2012; Tutu et al., 2020). In the articles under review, 3 to 16 statements were used to evaluate food safety practices among school children. Food can become contaminated at any stage of the food handling process. The risk factors include improper time/temperature control, improper food handling practices, inadequate cooking, contaminated raw foods and poor personal hygiene etc. (Medeiros, Hillers, Kendall, & Mason, 2001). The extent to which safe food handling practices are followed, during food purchasing, handling and preparation determines the probability of such events taking place. Participants in some studies often reported some risky behavioural approaches to preventing cross-contamination, storing and reheating leftovers in day-to-day life and personal hygiene (e.g., cooking in the presence of open wounds in hands and washing hands after using the washroom) (Abushelaibi et al., 2016; Ovca et al., 2014). In most cases, these risks can be eliminated, by following proper food handling practices (Fischer & Frewer 2009).

3.7 Limitations and future perspectives

These types of studies can be associated with limitations such as non-random selection of schools when getting the contribution of children to carry out the study. Less demographically diverse samples drawn from the population of interest is another limitation (Mullan, Wong, & Kothe, 2013). This may act as a hindrance when it comes to the generalization of the results of the study for the entire population in the whole territory (Cheng et al., 2017). In this regard, many researchers tend to consider how different sampling approaches can broaden the socio-demographic diversity of the samples taken for future studies. Meanwhile, for researchers dealing with the assessment of self-reported practices, there is a bias in the participants to typically provide more favorable behaviours (Cheng et al., 2017; Osaili et al., 2011). In such cases, researchers often apply strategies like emphasizing the participants that are being anonymized, and no individual evaluation will be done via participating in the study. As suggested by research work carried out by Levine, Chaifetz and Chapman (2017), the need for instrument validation of the survey study should be emphasized through increased testing and observations.

4. Conclusions

Studies regarding food safety KAP of school children are of utmost importance as they are capable of predicting the aspects of food safety in which improvements in knowledge, attitude, and practice are necessary. Further such studies can also

make a positive impact on food safety awareness among children. The assessment of food safety KAP of children should not limit to just answering a questionnaire, but it should follow a continuous process from evaluating them (through a survey) up to improving their knowledge (targeting under-developed areas of food safety) via proper education. Almost all the studies reviewed here lack the consideration of psychological factors which may affect their attitude and practices while handling food. Therefore, future research studies can focus on such areas to get more meaningful insights about food safety KAP among school children.

Acknowledgements

None.

Conflict of Interest

The authors of the present work do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

ORCID

Piyumi Chathurangi Wanniarachchi: https://orcid.org/0000-0003-3364-6433

Piumi De Abrew Abeysundara: https://orcid.org/0000-0003-3656-2462

Ethical Approval

Not applicable.

Publication Ethics

The submitted manuscript has not been previously published or is under review by another print or online journal or source.

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Available online at https://li02.tci-thaijo.org/index.php/ssstj

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Bacterial Contamination of Labor Wards and Delivery Rooms from Selected Primary Healthcare Facilities in Abia State

Mary Uche Kalu, Emmanuel Onwubiko Nwankwo, Ebubechi Uloma Okey-kalu*

Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, P.M.B 7267, Umuahia, Abia State, Nigeria

***Corresponding author e-mail**: ulomamgbeokwere@yahoo.com

Received: *22 August 2022* / **Revised**: *28 September 2022* / **Accepted**: *2 November 2022*

Abstract

Bacterial contamination of the labor and delivery room is of clinical concern because it is one of the major risk factors of sepsis in neonates and most life threatening nosocomial infections for mothers after undergoing childbirth procedures. From six different Primary Healthcare Centres (PHC), 300 samples of fomites were taken. They were screened for the presence of bacterial pathogens. Preliminary identification of bacterial isolates was performed based on Gram stain reactions and standard microbiological methods. Antibiotic susceptibility testing was done using Kirby-Bauer disc diffusion technique. The isolates of clinical importance observed were *Staphylococcus aureus* (35.1%), *Bacillus* spp. (15.5%), *Streptococcus* spp. (14.8%), *Escherichia coli* (10.1%), Coagulase Negative *Staphylococcus* (CONS) (8.1%), *Proteus* spp. (7.4%), *Pseudomonas aeruginosa* (5.4%), *Klebsiella* spp. (1.3%). Eight (8) antibiotics used against *Staphylococcus aureus* which was the most prevalent isolate showed below 50% sensitivity. High level resistance to commonly prescribed and administered antibiotics was observed. The most frequently isolated bacteria in this study were consistent with the isolates which could cause nosocomial infections.

Keywords: Bacterial isolates, Antibiotic, Susceptibility, Primary Healthcare Centers

1. Introduction

Today the increasing number of antenatal deliveries and the effect of the hospital environment on women's birth experience have become critical issues. Hospital environments that prioritize medical safety come about as a result of the movement of labor from the home to the hospital and the labeling of birth as a pathological event (Omo-Aghoja, Aisien, Akuse, Bergstrom, & Okonofua, 2010).

Babies especially those who are premature or have low birth weight, lack effective structural barriers, protective endogenous microbial flora, and a fully developed immune system at birth (Omo-Aghoja et al., 2010). The newborn represents one of the pediatric population's most vulnerable groups, particularly neonates delivered in contaminated primary healthcare facilities, where frequent use of medical equipment and immature immune systems increase the risk of nosocomial infections (Nevalainen et al., 1993).

Nevertheless, nosocomial infections remain a major cause of morbidity and mortality in developing countries where infection rates are relatively high with poor infection control practices, lack of supervision and inappropriate use of limited resources (Weinstein & Hota, 2004). Neonatal infection's main pathogens vary not just between nations and nurseries, but also change within years in the same nursery.

Healthcare workers not only contaminate their hands after direct patient contact but also after

touching inanimate surfaces and equipment in the labor ward zone (the patient and her immediate surroundings) (Hamza, 2010). Inadequate hand hygiene before and after entering a labor zone may result in cross-transmission of pathogens and patient colonization or infection. A number of equipment items and commonly used objects in labor wards carry bacteria which, in most cases, show the same antibiotic susceptibility profiles of those isolated from patients (Hamza, 2010). This study will present new findings regarding the microbial contamination of inanimate objects and equipment in labor and delivery rooms.

The main aim of this study is to investigate the Bacterial contamination that is associated with labor ward environment in some primary health care (PHC) setting within Umuahia metropolis.

2. Materials and Methods

2.1 Study location

Six public primary healthcare center (PHC) with high caseloads of pregnant women were selected from the twenty public primary health facilities within the Umuahia North Local Government Area. These Primary Healthcare centers were Nkwoegwu PHC, Orieogwu PHC, Azueke PHC, Ndume PHC, Lodu PHC and World Bank PHC.

2.2 Sample collection and processing

Sterile cotton swab sticks were prepared by making the cotton end wet with physiological saline. These were used to swab various items in the labor and delivery room. To ensure maximal coverage of a surface area, the swab were rolled back and forth over each surface.

From 22 sources in six (6) different labor wards of the Primary Health Care (PHC) Settings within Umuahia North Local Government Area of Abia State, a total of 300 consecutive samples were collected. Of these 300 samples, 270 samples were collected from sources in the six labor rooms namely; Operating Lamp (OPL), Floor (FL), Wall (WL), Sink (SK), Suction tube (ST), Forceps (FC), Scissors (SC), Trolley (TR), Weighing machine (WM), the couches/beds, tables, light switch, chairs, beds, door/locker handlers, trolley, stretchers, sinks/faucets, intravenous stands, and oxygen cylinder. All samples were labeled properly and transported to Microbiology laboratory within 30 minutes for microbiological analysis.

Following collection, the swabs were inoculated into MacConkey agar, Blood agar and manitol salt agar (Oxoid Limited). The agar plates inoculated were incubated at 37°C for 24 hrs and the growth was inspected to identify the bacteria. Preliminary identification of bacteria was performed based on gram stain reactions, colony characteristics of the organisms like hemolysis on blood agar, changes in physical appearance in differential media and enzyme activities of the organisms (Cheesbrough, 2000).

2.3 Antibiotic susceptibility test

Following isolation and identification, the microbial isolates were subjected to antibiotic sensitivity testing using the disc diffusion techniques described by Bauer, Kirby, Sherris and Turck (1966). The following antibiotics were used: ceftriaxone (30 µg), septrin (30 µg), cefalexin (30 µg), amoxicillin clavulanate (30 µg), gentamicin (10 µg), ofloxacin (10 µg), ciprofloxacin (5 µg), amoxicillin (10 µg), reflacine (30 µg), ampicillin (10 µg), streptomycin (30 µg), ampiclox (10 µg), cefuroxime (5 µg), levofloxacin (5 µg) and erythromycin (10 µg).

The examination of the control and test plates were carried out after overnight incubation to ensure the growth is confluent or near confluent. Using a ruler on the plate's buttom, the diameter of each zone of inhibition was measured in millimeter.

3. Results

A total of 300 samples were collected and analyzed from labor wards and delivery rooms of 6 primary healthcare centers of which 148 yielded bacterial growth. Ten different bacterial pathogens were identified. The organisms isolated with their percentage of occurrence were *Staphylococcus aureus* 52 (35.1%), *Bacillus* spp. 23 (15.5%), *Streptococcus* spp. 22 (14.8%), *Escherichia coli* 15 (10.1%), Coagulase Negative *Staphylococcus* (CONS) 12 (8.1%), *Proteus* spp. 11 (7.4%), *Pseudomonas aeruginosa* 8 (5.4%), *Klebsiella* spp. 2 (1.3%), *Enterobacter* spp. 2 (1.3%) and *Micrococcus* spp. 1 (0.6%). The isolates showed below 50% sensitivity to a range of commonly prescribed and administered antibiotics such as amoxicillin, septrin, amoxicillin clavulanate, ceftriaxone and ampicillin.

Table 1. Frequency and distribution of bacterial isolates.

Isolates	No	Percentage (%)
S. aureus	52	35.1
Strept. spp.	22	14.8
CONS	12	8.1
Enterobacter spp.	2	1.3
E. coli	15	10.1
Pseudomonas aeruginosa	8	5.4
Proteus spp.	11	7.4
Klebsiella spp.	2	1.3
Bacillus spp.	23	15.5
Micrococcus spp.	1	0.6
Total	148	100

CONS – Coagulase Negative *Staphylococcus*

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Table 2. Total distribution pattern of the bacterial isolates from fomites.

ITEMS	*S. aureus*	*Strept* spp.	CONS	*Enterobacter* spp.	*E. coli*	*Pseudomonas aeruginosa*	*Proteus* spp.	*Klebsiella* spp.	*Bacillus* spp.	*Micrococcus* spp.	Total No of Isolates (%)
Beddings	14	4	6		6	2	1		8		41 (33.8%)
Baby cot	1	1			1			1		1	5 (4.1%)
Bowel	1	1	1		1	2	5		1		12 (9.6%)
B.P app		1									1 (0.8%)
Weighing Machine								1			1 (0.8%)
Trolley	1	1							1		3 (2.4%)
Sink				1	1	1					3 (2.4%)
Floor	2			1	1		1		5		10 (8.2%)
Chair	8	4	1		1				4		18 (14.5%)
Lamp									1		1 (0.8%)
Cupboard			2			1			2		5 (4.1%)
Drawer	1										1 (0.8%)
Curtain	1				1						2 (1.6%)
Switch	2						1				3 (2.4%)
Drip stand	2	1					1				4 (3.3%)
Bed pan	2					1	1				4 (3.3%)
Forcep	1	1									2 (1.6%)
Scissors	1	1				1					3 (2.4%)
Gali pot		1							1		2 (1.6%)
Pillow		1									1 (0.8%)
Bathtub		1					1				2 (1.6%)
Isolates	37 (29.3)	18 (14.5)	10 (8.0)	2 (1.6)	12 (9.6)	8 (6.4)	11 (8.0)	2 (1.6)	23 (18.5)	1 (0.8)	124 (100%)

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Table 3. Antibiotic susceptibility of bacterial isolates.

		No of isolates sensitive to = N (%)											
Gram (-) Isolates	**Total No**	**PEF**	**CN**	**Z**	**AM**	**CRO**	**CPX**	**S**	**SXT**	**OFX**	**AU**	**PN**	**CEP**
Enterobacter spp.	2	2 (100)	2 (100)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	1 (50)	0 (0)	0 (0)
E. coli	15	10 (66)	10 (66)	3 (20)	3 (20)	4 (26)	9 (60)	10 (66)	1 (6)	11 (73)	4 (26)	1 (6)	0 (0)
Psuedomonas aeruginosa	8	3 (37)	4 (50)	0 (0)	0 (0)	0 (0)	3 (37)	5 (62)	0 (0)	4 (50)	2 (25)	1 (12)	1 (12)
Proteus spp.	11	3 (27)	6 (54)	3 (27)	3 (27)	3 (27)	5 (45)	6 (54)	2 (18)	6 (54)	2 (18)	2 (18)	1 (9)
Klebsiella spp.	2	1 (50)	2 (100)	0 (0)	0 (0)	0 (0)	2 (100)	2 (100)	2 (100)	2 (100)	1 (50)	2 (100)	2 (100)

		No of isolates sensitive to = N (%)											
Gram (+) Isolates	**Total No.**	**PEF**	**CN**	**APX**	**Z**	**AM**	**CRO**	**CPX**	**S**	**SXT**	**E**	**OFX**	**LEV**
S. aureus	52	36 (62)	23 (44)	7 (13)	38 (73)	13 (25)	21 (40)	27 (51)	37 (71)	17 (32)	27 (51)	31 (59)	22 (42)
Strept. spp.	22	13 (59)	10 (45)	10 (45)	14 (63)	10 (45)	13 (59)	13 (59)	9 (40)	10 (45)	11 (50)	11 (50)	15 (68)
CONS	12	5 (41)	6 (50)	5 (41)	5 (41)	6 (50)	5 (41)	8 (66)	9 (75)	6 (50)	7 (58)	0 (0)	8 (66)

OFX-Ofloxacin, PEF-Reflacine, CPX-Ciprofloxacin, AU-Augmentin, CN-Gentamycin, S-Streptomycin, CEP-Ceporex, SXT-Septrin, PN-Amplicin, Ampicillin, E-Erythromycin, CH-Chloramphenicol, APX-Ampiclox, LEV-Levofloxacin, Z- Zinnacef and CRO-Ceftriaxone, AM- Amoxycillin

3. Discussion

Numerous bacteria have been found to be abundant in hospital environments, and contaminated surfaces have been shown to increase the occurrence of healthcare- associated infections, especially in the most vulnerable age groups (Infancy), where immunity is decreased (Orji, Mbata, & Kalu, 2005).

A total of 300 swab samples were collected from various surfaces in the labor/delivery rooms of the six (6) different PHCs. Of the 300 swab samples collected from various surfaces 148 (49.3%) were positive for bacterial growth. This is similar to the results obtained in Maiduguri by Okon et al. (2012) who sampled 267 hospital surfaces: 50.0% were positive for bacterial growth.

The predominant bacterial contaminant in this study was *Staphylococcus aureus* accounting for 35.1% of the organism isolated. This was similar to the findings of a study carried out in Sokoto, where *Staphylococcus aureus* was equally the most prevalent isolate (Saka et al., 2016). The higher prevalence of *Staphylococcus aureus* may be due to ubiquitous distribution in human body as part of the normal flora (normal microbiota) of the anterior nares, nasopharynx and the skin (Forbes, Sahm, & Weissfeld, 2007). *Staphylococcus aureus* has pre dilection for inanimate surfaces and are relatively resistant to drying, heat and sodium chloride, these properties allow its survival on inanimate surfaces. The predominance of *Staphylococcus aureus* as found in this study is contrary to the findings of Okon et al. (2012) whose predominant isolates was found to be CONS and also at variance to the work of Garcia-Cruz, Najera Aguilar and Arroyo-Helguera (2012) in Mexico, who reported a high prevalence of *Klebsiella* spp.

The source of CONS from this study could include the normal skin flora (microbiota) of medical personnel, patients and fabrics (Cheesbrough, 2000). However, clinical implication of CONS is more pronounced in immunocompromised patients, as entry into systemic environment could initiate infections, and some of the expectant mothers attending these PHCs are HIV positive. Although not considered a pathogen, *Bacillus* spp. was the second most common isolate in this study. The findings of Singh, Kaur, Gardner and Treen (2002), Gebremariam and Declaro (2014) showed this same organism as the most frequently isolated in their studies.

In this study, the most frequently bacterial isolates was coagulase negative *Staphylococcus* spp. followed by *Pseudomonas aeruginosa*. The same observation was reported by Ensayef, Al-Shalchi and Sabbar (2009) and Onwubiko and Akande (2015) although they did not state the proportion in their reports. It is known that both bacteria easily acquire resistance to antibiotics and can cause both superficial and fatal systemic infection (Turner & Craddock, 1973). *P. aeruginosa* has the ability to survive and spread in hospital environments as a result of acquisition of multiple virulence determinants and intrinsic resistance to commonly used antibiotics and also disinfectants (Turner & Craddock, 1973). This is why *Pseudomonas. aeruginosa* is regarded as a major life threatening agent that is responsible for many outbreaks of nosocomial infections (Orji et al., 2005).

The antimicrobial susceptibility pattern of clinically relevant pathogens tested, showed similar pattern with high resistant level to amoxicillin and ampicillin, moderate resistance to erythromycin, and ciprofloxacin and high sensitivity to streptomycin, ofloxacin and gentamycin.

4. Conclusion

This study confirmed that various surfaces in the delivery room were contaminated with known bacterial pathogens. Direct involvement of these surfaces in disease transmission was not investigated in this study, but the isolation of Coagulase Negative *Staphylococcus* (CONS), *Pseudomonas aerugionosa*, *Proteus* spp., *Streptococcus* spp., *Staphylococcus aureus*, including *E. coli* presents a serious concern for possible nosocomial transmission.

This study also showed high degree of bacterial load that is beyond the standard limits on both surfaces of the hospital. Therefore, it is important to evaluate and strengthen the infection prevention practice of the primary health care centers within the Umuahia metropolis. Moreover, stakeholders should also reinforce actions to decrease the pressure of antimicrobial resistance in this studied area.

Acknowledgement

We appreciate the staff and management of Umuahia North Local Government Area primary healthcare centers for their support during the course

of this research and the Abia State Primary Healthcare Agency Research Ethics Committee for approving this study. We appreciate Prof. Uchenna Ota for his financial contributions.

Conflict of Interest

The author's do not report any financial or personal connections with anyone.

ORCID

Author 1: Mary Uche Kalu
https://orcid.org/0000-0002-5738-7934
Author 2: Emmanuel Onwubiko Nwankwo
https://orcid.org/0000-0002-4025-0886
Author 3: Ebubechi Uloma Okey-kalu
https://orcid.org/0000-0002-4872-3801

Ethical Approval

This study was approved by the Abia State Primary Healthcare Agency Research Ethics Committee. (Date: 10th December, 2017). Number of ethics: (RP/REC/2017/338).

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Available online at https://li02.tci-thaijo.org/index.php/ssstj

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Bacterial Contamination of Door Handles of Commercial Buses in Umuahia Metropolis Abia State

Emmanuel Onwubiko Nwankwo*, Ebubechi Uloma Okey-kalu,

Favour Amuche Eze

Department of Microbiology, College of Natural Sciences,
Michael Okpara University of Agriculture Umudike,
P. M. B 7267, Umuahia, Abia State, Nigeria
***Corresponding author e-mail**: emmaonwubiko@yahoo.com

Received: *4 August 2022* / **Revised**: *28 September 2022* / **Accepted**: *2 November 2022*

Abstract

Commercial vehicles are all kinds of vehicles used to transport goods and people. Microbiological investigation of publicly touchable surfaces has become an interest to researchers because surfaces that are contaminated can function as reservoir of potential pathogens. This study was carried out to determine the levels of bacterial contamination on the surfaces of two door handles on public buses within Umuahia metropolis. A total of 70 samples, 35 each from the passengers and driver's door handles surface were collected and analyzed. The analysis involved bacterial isolation and identification of organisms by standard microbiological procedures. The prevalence and bacteria count were carried out. The antibiotic sensitivity pattern of the isolates were done using the disc diffusion method. A total number of 73 bacterial organisms were isolated. The following potential pathogenic bacteria were observed; *Staphylococcus aureus* (6.8%), *Staphylococcus epidermidis* (5.5%), *Streptococcus faecalis* (2.7%), *Escherichia coli* (6.8%), *Klebsiella* spp. (5.5%), *Enterobacter* spp. (4.1%), *Pseudomonas aeruginosa* (17.8%), *Proteus* spp. (23.3%), Micrococcus spp. (6.8%) and *Bacillus* spp. (20.5%). Ofloxacin, Pefloxacin and Gentamicin are effective and exhibited encouraging results while Ampicillin and Trimethoprim were resistant to most isolates. The result actually shows that the door handles of motor vehicle used for public transportation can be a significant reservoir for spreading potential pathogenic microorganisms.

Keywords: Bacterial contamination, Commercial buses door handles, Antibiogram

1. Introduction

There is a misconception that microbes are only found in research laboratories and healthcare facilities. Causes of health problems could be as a result of lack of knowledge about where germs grow. About 80% of infections are spread through direct contact with hands (Al-Ghamdi et al., 2011; Reynolds, Watt, Boone, & Gerba, 2005). Microorganisms that cause infections can be found in any environment including soil, air, water, food and other organisms as well as on environmental surface or objects. The spread of infectious diseases due to contact with the hands is a major concern. Hand washing that is well known and the first thing that is done in preventing diseases from spreading has been abandoned and must be enhanced vigorously by families, schools and healthcare professionals.

The ability of inanimate objects to support viable microorganisms for a long period of time is well documented (Fierer, Hamady, Lauber, & Knight, 2008) and such environmental surfaces and objects especially those in proximity with persons and frequently touched pose a threat to human health and area cause for concern. Also recent studies have shown the presence of bacterial pathogens on hard, non-porous surfaces such as kitchen surfaces, floor surfaces, toilet surfaces, door handles etc. (Maori, Agbor, & Ahmed, 2013; Nworie, Ayeni, Eze, & Azi,

2012) from which pathogens are easily transmitted to unsuspecting members of the public posing potential risk to vulnerable, immune-compromised individuals (Fierer et al., 2008). Currently, some of these bacterial pathogens have become antibiotic resistant, which is a major public health crisis facing the world today (Ventola, 2015; Voicu et al., 2017). Chittagong and many other cities of the country lack adequate transportation. Exorbitant prices of other forms of private transportation and inadequate provision of railroads in the city have left people with no option than to use public bus. Public buses are rarely cleaned and focus only on sweeping of floors and windows even if cleaned (Yeh, Simon, Millar, Alexander, & Franklin, 2011). Microbial load on touch surfaces are built up by the unhygienic and humid condition of buses and may lead to mild to severe infection in human from such reservoirs through interpersonal transfer of microorganisms.

Nworie et al. (2012) carried out a research on 180 door handles/knobs of public toilets of public offices, motor parks and markets that were selected in Abuja Metropolis, Nigeria. It was discovered that 156 (86.7%) yielded bacterial growth. The bacterial isolates were *Staphylococcus aureus* (30.1%), *Klebsiella pneumoniae* (25.7%), *Escherichia coli* (15.6%), *Enterobacter* spp. (11.2%). *Citrobacter* spp. (7.1%), *Pseudomonas aeruginosa* (5.9%) while *Proteus* species was the least isolated (4.5%).

Sabra (2013) Investigated on public female restrooms at Taif, Kingdom of Saudi Arabia, in order to know the level of contamination and bacterial loads. It was discovered that out of the 260 specimens, 187 (71.9%) turned out to be positive. Toilet handles (91.3%) had a higher percentage followed by room door handles 59 (73.8%), and room sink 38 (63.3%). Bacterial isolates in order of how they were frequently isolated are; *S. aureus* 40.6%, *E. coli* 22.5%, *Bacillus* spp. and *K. pneumoniae* 21.4%, *Enterococcus faecalis* 13.4%, *Citrobacter* spp. 9.6%, *P. aeruginosa* 8.6% and *Proteus mirabilis* 7%.

The aim of this study was to evaluate the potential bacterial pathogens as contaminants on commercial bus door handles.

2. Materials and Methods

2.1 Study Area

This study was conducted in Umuahia metropolis, Abia State in the South-East of Nigeria between April to July 2021. Samples was collected from three different locations as stated below:

Sample A was collected in MOUAU School Park, sample B was collected in Isi-Gate, while sample C was collected at Cooperative. All samples were properly labeled and transported to the Microbiology laboratory for examinations.

2.2 Sample collection and processing

Sample was collected by swabbing the door handle with a sterile cotton swab stick soaked in sterile water from each location aseptically. Each sample was taken twice per bus randomly and were transported to the laboratory for analysis (Cheesbrough, 2006).

Serial dilution was done from the soaked sample collected from the three different locations in Umuahia metropolis. In the procedure, 9ml of normal saline was poured into 5 different sterile test tubes labelled 10-1, 10-2, 10-3, 10-4, and 10-5. For each of the sample collected, 2ml of normal saline was added and shaken, I ml was transferred into the first test tube labeled 10-1 and tilted gradually to mix, after that 1ml from it was taken into the second test tube labeled 10-2 and from it I ml was taken after shaking to mix to the test tube labeled 10-3 until the last dilution factor i.e. 10-5 where after mixing I ml was discarded. Serial dilution was done throughout the project processes for each of the sampled collected. All the available samples were processed for microbial isolation in a sterile atmosphere, by swabbing the work bench and lightning a spirit lamp on the table. The following culture media were used: MacConkey agar, Nutrient agar, Mannitol salt agar and Blood agar were used. The samples were inoculated on the plates of the different prepared media aseptically using streak method and incubated for 24hours at 37°C. Pure colonies of isolate organisms were identified and characterized using standard microbiological techniques (Cheesbrough, 2006).

2.3 Antibiotic susceptibility test

Antibiotic disc sensitivity testing was carried out on each of the bacterial Isolates with the Mueller-Hinton agar. Standard paper discs soaked with adequate amount of antibiotics were placed in the Mueller-Hinton agar in this method. After Incubation for 24 hours, antibiotic activity was determined by zone of Inhibition, (No growth)

around the antibiotic disc and was measured in millimeters (mm) with a transparent meter rule and a protractor. The susceptibility was carried out for the following antibiotics; Ofloxacin (10µg), Peflacine (10µg) Ceftriaxone (10µg), Amoxicillin (30µg), Gentamycin (10µg), Ciprofloxacin (5µg). Amoxicillin/Clavulanate (10µg) and Ampicillin (30µg). With the aid of 0.1ml of 0.5 McFarland's standard as a turbidity check for a semi confluent growth, these antibiotics were tested after an overnight cultures of the isolates on Mueller-Hinton agar. With reference to the Clinical and Laboratory

Standard Institute (CSL1) performance standard for anti-microbial susceptibility, the organism's susceptibility or resistance pattern to the drugs used was done.

3. Results

In the course of this investigation, a total of 70 samples, 35 from the driver's door handle side and 35 from the passenger's door side were aseptically collected with a sterile swab stick, labeled properly and transported to the laboratory for analysis.

Table 1 shows all the bacterial isolates and their percentage. A total of 61 bacterial organisms were isolated. These organisms comprised of nine (9) genera out of which *Proteus* spp. (23.3%) and *Bacillus* spp. (20.5%) ranked the highest in frequency of occurrence.

Table 2 shows the mean bacterial load of isolates from the passenger and driver's side. Antibiotic pattern of the isolates is shown in Table 3. The result indicates that most of the isolates were sensitive to the tested antibiotic.

Table 1. Prevalence of various isolates observed in the study.

Isolates	No isolated	Percentage (%)
Pseudomonas aeruginosa	13	17.8
Proteus species	17	23.3
Klebsiella species	4	5.5
Micrococci	5	6.8
Staphylococcus epidermidis	4	5.5
Bacillus species	15	20.5
Escherichia coli	5	6.8
Staphylococcus aureus	5	6.8
Streptococcus faecalis	2	2.7
Enterobacter spp.	3	4.1
TOTAL	**73**	**100**

Table 2. Mean bacterial load of isolates (cfu/cm²) from the passenger and driver's side.

Isolates	Passenger	Driver
Pseudomonas aeruginosa	$9.0\times10^{3}\pm3.4$	$5.0\times10^{2}\pm1.4$
Proteus species	$4.0\times10^{4}\pm2.3$	$3.0\times10^{3}\pm2$
Klebsiella species	$7.0\times10^{4}\pm1.1$	$4.0\times10^{4}\pm2.3$
Micrococci	$5.0\times10^{3}\pm3.1$	-
Staphylococcus epidermidis	$6.3\times10^{2}\pm3.2$	$2.0\times10^{2}\pm2$
Bacillus species	$4.0\times10^{4}\pm2.1$	$3.4\times10^{3}\pm2.3$
Escherichia coli	$8.0\times10^{4}\pm2.3$	$3.5\times10^{5}\pm3$
Staphylococcus aureus	$6.0\times10^{3}\pm5.2$	$4.I\times10^{3}\pm2.5$
Streptococcus faecalis	$5.0\times10^{2}\pm1.5$	$2.3\times10^{4}\pm2.1$
Enterobacter spp.	$7.0\times10^{3}\pm2.2$	-

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Table 3. Antibiotics susceptibility pattern of the isolates from various motor vehicle door handles.

		Number sensitive (%)								
Organism	**No Tested**	**OFX**	**PEF**	**CN**	**AU**	**S**	**SXT**	**PN**	**CPX**	**CEP**
P. aeruginosa	15	8 (61.5)	7 (53.8)	6 (46.1)	1 (7.7)	6 (46.1)	0 (00)	0 (00)	8 (61.5)	3 (23.0)
Proteus species	17	14 (82.3)	12 (70)	10 (58.8)	4 (23.5)	5 (29.4)	2 (11.8)	2 (11.8)	10 (58.8)	4 (23.5)
Klebsiella species	4	4 (100)	3 (75)	0 (00)	2 (50)	2 (50)	0 (00)	0 (00)	2 (50)	1 (25)
Micrococci	5	-	-	-	-	-	-	-	-	-
S. epidermidis	4	1 (25)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)
Bacillus species	15	-	-	-	-	-	-	-	-	-
Escherichia coli	5	3 (60)	3 (60)	3 (60)	3 (60)	3 (60)	0 (00)	0 (00)	0 (00)	1 (20)
S. aureus	5	3 (60)	3 (60)	2 (40)	1 (20)	2 (40)	0 (00)	0 (00)	1 (20)	1 (20)
S. faecalis	2	0 (00)	1 (50)	0 (00)	1 (50)	0 (00)	1 (50)	0 (00)	0 (00)	0 (00)
Enterobacter spp.	3	0 (00)	0 (00)	2 (66.7)	1 (33.3)	1 (33.3)	0 (00)	0 (00)	0 (00)	0 (00)

Key SXT=Trimethoprim, CN= Gentamicin, AU=Amoxicillin/ Clavulanate, OFX=Ofloxacin, PEF= Pefloxacin, PN=Ampicillin, CEP= Cephalexin, S= Streptomycin, CPX= Ciprofloxacin

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4. Discussions

Bacterial contamination of door handles is well documented and these fomites act as pathway for cross-infections. Some of the contaminants can be highly pathogenic and can be transferred directly or may result in auto-inoculation (Kennedy, Enriquez, & Gerba, 2007).

The surfaces of commercial bus door handles usually come in contact with the palms of various individuals of different hygienic backgrounds. Evaluation of possible bacterial contaminant is targeted at the isolation and identification of potential pathogens which could cause infection in man when the palms are contaminated. A total of 70 door handles swabs from different commercial bus of the passenger and driver side in Umuahia metropolis were investigated to ascertain the level of bacterial contamination and to identify the bacteria contaminants in this study. Out of 70 samples assessed, 62 (88.6%) of them yielded bacterial growth. Some of the positive samples examined had more than one type of bacterial isolate. This result compares favorably with that obtained by Nworie et al. (2012) in Abuja Metropolis, Nigeria, who reported that out of 180 door handle swabs assessed, 156 (86.7%) showed bacterial growth, and also in agreement with reports of Onwubiko and Chinyeaka (2015) who observed 86 (86%) bacterial contamination of door handle out of 100 samples assessed in MOUAU.

The outcome of this research showed that *Staphylococcus aureus*, *Bacillus* spp., *Micrococcus* spp., *Escherichia coli*, *Proteus* spp., *Streptococcus faecalis*, *Staphylococcus epidermidis*, *Enterobacter* spp., *Pseudomonas aeruginosa* and *Klebsiella* spp. are the main bacterial isolates associated with the door handles frequently as also recorded by Bashir, Muhammad, Sani and Kawo (2016) whose result shows to be *Staphylococcus aureus*, *Bacillus* spp., *Micrococcus* spp., *Escherichia coli*, *Salmonella* spp. and *Klebsiella* spp. are the main bacterial isolates always associated with toilet door handles. Through the skin and direct contact, these organisms may probably have their way to the door handles.

In the current study, both Gram negative and Gram positive bacteria were isolated. This is at variance with the reports of Datta, Rani, Chander and Gupta (2009) who isolated only Gram positive bacteria: *Staphylococcus*, *Enterococcus*, *Micrococcus* and *Streptococcus* from mobile phones and in agreement with the studies of Yazah, Yusuf and Agbo, (2012), and Cataño, Echeverri and Szela (2012) who isolated Gram positive and Gram negative bacteria in their research from currency note and computer keyboards, curtains, cellphones, white coats and ties.

The findings from this research reveals *Proteus* spp. and *Bacillus* spp. to be the most frequently bacterial isolates with a percentage occurrence of 23.3% and 20.5% respectively with the viable count being higher in the passenger's side when compared with the driver's side due to the number using the handle is higher. This is in agreement with finding of Onwubiko and Chinyeaka (2015) who reported that in canteens, female restroom, male and female hostels, the level of contamination was higher in that order as compared to classrooms and laboratory that were low. Laboratory and classrooms showed a lower level of contamination and it could be attributed to the fact that they are not always in use as other places studied. This is also in agreement with the findings of Boone and Gerba (2010) and Nworie et al. (2012) who reported that depending on the exposure, environment and traffic, the level of contamination varies.

A high percentage of gram-negative rods isolated in this study which includes *Proteus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella* spp. indicate the possibility of the presence of faecal contamination on the door handles. This may be as a result of contaminated hands with fecal and urinary material while using the toilets. The issue of hygiene is taken with levity which result in failure to wash their hands and the concept of hand washing as a common means of stopping the spread of these infectious agents are lacked. This compares favorably with the work of Zhad, Zhad, Doyle and Meng (1998), who reported that the high rate of isolation of these organisms in which human hands serve as means of transmission is only obtained during epidemics. Gram negative sepsis, urinary tract infections are most commonly caused by *E. coli*, *Klebsiella* spp. and *Proteus* spp. Also a high percentage of *Bacillus* spp. a gram positive organism isolated could be explained by the fact that *Bacillus* spp. are everywhere with its ability to form spores that can withstand harsh environmental condition, withstand dry heat and certain chemical disinfectants for extended period of time (Onwubiko & Chinyeaka, 2015). This is also in agreement with the work of

Brooks, Carroll, Butel and Morse (2007) who reported that *Bacillus* spp. was discovered to be a few of the primary organism that was isolated from door handles, Ashgar and El-Said (2012), also reported the isolation of *Bacillus* spp. from environmental sites in Mecca city.

Staphylococcus aureus cause boils, toxic shock syndrome, pneumonia, abscesses, wound infections and other disease (Yusha'u, Bello, & Sule, 2010) while *Staphylococcus epidermidis* is responsible for endocarditic and infections of patients with low immunity. The presence of these organisms indicate that the use of public buses, public motorcycle, ATMs and door handles can lead to circulation of serious skin infections.

Streptococcus A strains are found in the throat and skin and can cause strep throat and impetigo, a common skin infection that primarily affects children. The manifestation of *Enterobacter aerogenes* and *E. coli* shows likely faecal contamination of the ATMs, public toilets and motorcycle and the consequence could be a potential source of food poisoning when eating with infected hands and preparing food without proper hand washing (Yusha'u et al., 2010).

Antibiotics susceptibility studies of the isolates showed a pattern of resistance to commonly used antibiotics such as Trimethoprim, Ampicillin and Gentamicin. This is in agreement with the reports of Onwubiko and Chinyeaka (2015), and Nwankwo and Offiah (2016) who reported that antibiotic resistant microorganism contaminates environmental surfaces such as door handles in a tertiary institution in Umuahia and interface of automated teller machine (ATM) in Umuahia metropolis respectively. The isolates recorded in their research were resistant to commonly used antibiotics such as Cotrimoxazole, Amoxicillin/Clavulanate, Gentamicin and Ampicillin.

5. Conclusion

The results of this study show that several bacteria often occupy public contact area such as door handles and function as a possible source of infections.

Regular cleaning of door handles by drivers and hand washing by the passengers could reduce contamination as a result of transmission of these bacteria organisms.

Acknowledgement

We extend our gratitude to the operators of commercial buses in Umuahia Metropolis for their cooperation during the course of the research.

Conflict of Interest:

The author's do not report any financial or personal connections with anyone.

ORCID

Author 1: Emmanuel Onwubiko Nwankwo
https://orcid.org/0000-0002-4025-0886

Author 2: Ebubechi Uloma Okey-kalu
https://orcid.org/0000-0002-4872-3801

Author 3: Favour Amuche Eze
https://orcid.org/0000-0002-0709-2898

Ethical Approval

This study was approved by the National Union of Road Transport Workers {Umuahia branch} (NURTW/003/05/21). Date: 6th, February, 2021.

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Sound Insolation of Local Economic Materials Prepared by Natural Rubber Latex and Pineapple Leaf Fiber

Sonthaya Pongthanee, Nuorn Choothong*

Faculty of Engineering, Rajamangala University of Technology Rattanakosin,
96 Moo 3 Phutthamonthon, Nakhon Pathom 73170, Thailand
***Corresponding author e-mail: nuorn.cho@rmutr.ac.th**

Received: *15 May 2022* / **Revised:** *20 October 2022* / **Accepted:** *3 November 2022*

Abstract

The sound insulation properties of local economic materials made from natural rubber latex and pineapple leaf fiber were investigated, in order to develop and increase the economy of the community. The surface of pineapple leaf fiber was treated with alkaline treatment individually. The random fiber range was mixed with natural rubber latex in five different fractions, varying from 10% to 50% with an increment of 10%. The thickness of each sample is kept constant at 50 mm. Method used to measure sound absorption coefficient (α) is Impedance Tube Method ASTM E1050-09, which has been used for measuring the sound absorption and sound transmission loss. It was found that the sound absorption coefficient (SAC) increases with the fiber fraction. The sound insulation test results show that the obtained product of natural rubber with pineapple leaf fiber has a promising possibility that can be used as a potential material for the development of sustainable sound absorber material.

Keywords: Natural rubber latex, Pineapple leaf fiber, Sound insolation property

1. Introduction

Noise pollution is one of the world's points concerns as it affects human health and environment quality (Peng, 2017; Zhu, Kim, Wang, & Wu, 2014). In order to control the noise, sound barrier as an acoustic material is a trend to be investigated for use in building for sound isolation purposes. However, most of the acoustic materials currently are found to be unsustainable in terms of energy consumption, greenhouse gas emission and their impact on human health. For instance, mineral wool causes health impact (Abdul Latif et al., 2015; Asdrubali, 2007) and the synthetic materials such as synthetic fibers which are being widely used as acoustic material because of their high stiffness and strength properties (Rout, Misra, Tripathy, Nayak, & Mohanty, 2001). These synthetic materials also offer good acoustical performance. However, the synthetic fibers have serious disadvantages in terms of initial processing cost, recyclability, energy consumption and machine abrasion (Faruk, Bledzki, Fink, & Sain, 2012). Therefore, sustainable acoustic material, especially natural fiber which gained much attention in recent years under the sustainable development building agenda.

Among the natural fibers used in acoustic material, Pineapple leaf fiber is an alternative replacement for synthetic fiber in acoustical application because of its fiber properties. They have been reported to have high young's modulus and tensile strength (Asim et al., 2015), including its conductivity (Asdrubali, D'Alessandro, & Schiavoni, 2015) among other natural fibers. The fibers are well known to have high content of cellulose and thus can be used as the source of cellulose nanofibrils for biotechnology applications (Cherian et al., 2010).

Recently, the pineapple leaf was reported to be an alternative natural acoustic material (Putra et al., 2018). The different densities and thicknesses of the pineapple leaf fiber have been reported. From the sound absorption measurement, it revealed that the pineapple leaf fiber could achieve a sound absorption coefficient of 0.9 on average above 1 kHz by controlling the densities of the fibers. It is also demonstrated that the sound absorption performance is similar to that of the commercial rock wool fibers and synthetic polyurethane foam. However, there are some disadvantages of using pure natural fiber such as fire point mechanical properties and

moisture. Therefore, the fiber-reinforced composites were developed to improve its properties in order to use for interior sound absorber material.

The aim of this work was to prepare pineapple leaf fiber bond with natural rubber in various amounts of natural rubber latex which act as a binder; that was, 40% 30% and 20% of natural rubber latex. The sound absorption of the samples was performed by using the impedance tube method. The effect of density and porosity of the samples with various binder contents were investigated in order to improve the absorption at low frequencies.

2. Materials and Methods

2.1 Materials

Natural rubber latex with high ammonia was purchased from Sri Trang Agro-Industry Public Company Limited, Songkhla, Thailand. Pineapple leaf fiber was received from Tambon Nongplub, Amphoe Hua-Hin, Prachuapkhirikhan province [Figure 1]. In order to remove unwanted shells and dirt, the fibers were first dewaxed by extracting in 1:2 (V/V) mixture of ethanol and benzene for 72 h [Figure 2]. Then, the fibers were soaked in alkaline treatment at 5 wt% of NaoH for 72 hours to remove cellulose layer and unwanted properties of the law fibers. After that the soaked fibers washed with distilled water until pH 7. The fibers were dried in the oven at 50^{o}C for 24 hours. Sodium hydroxide, NaOH, sodium dodecyl sulfate, SDS was purchased from Carlo Erba reagent.

2.2 Method

2.2.1 The preparation of pineapple leaf fiber bound with natural rubber latex (PF/NRL)

The pineapple leaf fibers were bound together with natural rubber latex in various percentages of 40%, 30% and 20%. All the samples were fabricated at constant thickness of 50 mm by casting, following the standard thickness of synthetic acoustic panels that are available in the commercial market. Since the thickness has influence on the sound absorption coefficient of the material (Ismail, Ghazali, Mahzan, & Zaidi, 2010).

2.2.2 Bulk density

The specific density of the PL/NRL is determined from the principle of Archimedes**.** The weight of the dry fiber sample was measured and recorded by placing it on the weight tray, then, PL/NRL was placed into the water and the weight of the immerse fiber sample was recorded using equation (1).

Figure 1. Pineapple plant at a pineapple farm in Prachuapkhirikhan province, Thailand.

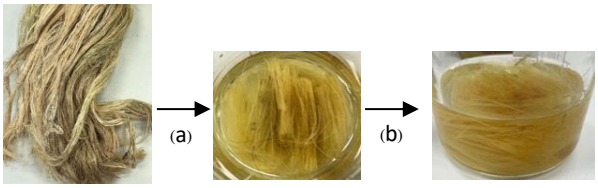

Figure 2. The extraction steps of pineapple leaf fiber following by dewaxation of the fiber (a) and alkaline treatment (b).

$$\rho = \frac{W_d}{W_d - W_s} - \rho_w \quad (1)$$

Where, W_d is the weight of a dry sample in grams**.** W_s belongs to the weight of the sample saturated in water and ρ_w is density of water**.**

2.2.3 Porosity

In sound absorption mechanism terms, porosity is an important factor that influences the sound absorption. Since there are many researchers who have declared the importance of porosity on calculating the acoustical absorptive properties of porous material (Biot, 1962). The measurement of the open porosity was performed using the same Archimedes principle as porosity indicator by using equation (2).

$$\emptyset = \frac{W_d}{W_s} \quad (2)$$

Sound absorption coefficient (SAC)

The acoustic analysis of pineapple leaf fiber bound with natural rubber latex (PL/NRL) was performed by Impedance tube following ASTM E1050. Sound absorption coefficient (SAC) is the fraction of sound energy absorbed by the materials, which represents a value ranging from 0 to 1. It is

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the ratio of absorbed energy to incident energy, represented by α in equation (3)

$$\alpha = 1 - \frac{E_r}{E_i} \quad (3)$$

Where Er is the reflected acoustic power and Ei is the incident acoustic power.

The sound absorption was tested using ASTM E1050-09, with the two-way microphone. This test method covers the use of an impedance tube, also known as standing apparatus, for the measurement of impedance ratios and the normal incidence sound absorption coefficients of acoustical materials.

3. Results and Discussion

Since the bulk density is a significant parameter that affects the sound absorption properties (Sreekala, Kumaran, Joseph, Jacob, & Thomas, 2000). The bulk densities of PF without and with NRL are tested and listed in Table 1. The results indicated that the density of pineapple leaf fiber was increased from 204.5 kg/m^3 to 773.5 kg/m^3 of pineapple leaf fiber bind with natural rubber latex at 40%. The increased pineapple leaf fiber content in natural rubber latex, the higher bulk density.

Table 1. The physical characteristics of pineapple leaf fiber bind with natural rubber latex.

Ratio PF/NRL (%)	Density (kg/m^3)	Porosity	Average Diameter (μm)
40	773.5	0.56	18.8
30	636.1	0.69	17.3
20	513.9	0.78	15.2
0	204.5	0.88	11.9

The porosity of PF/NRL was increased while the pineapple leaf fiber content was decreased (Table 1). The PF/NRL at 40% of binder showed the lowest porosity compared to PF/NRL at 30% and 20%. It is because the gab of fiber is less existing due to the higher percentage of binder cover up and dissolves in the pore and micro gaps inside the sample.

The surface morphology of treatment pineapple leaf fiber was analyzed by scanning electron microscope (SEM). Figure 3 shows SEM micrograph of treated pineapple leaf fiber with 5 wt% of NaOH. The treated fiber had a rough surface. Furthermore, the treatment also has an effect on the fiber, the amount of cellulose exposed on the fiber surface increased, and the surface roughness increased. This condition promotes better interlocking between binder and fiber. Moreover, the porous structure was observed in NaOH treatment. These porous structures bonded to the natural rubber latex that melted and interlock together.

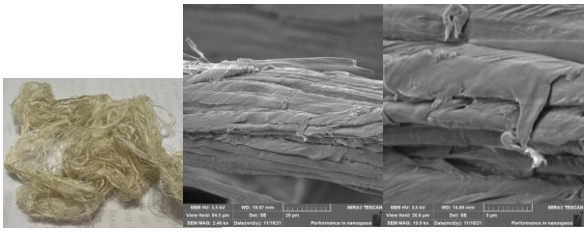

Figure 3. Picture image of the pineapple leaf fiber surface which treated with 5 wt% NaOH (a), Scanning electron micrographs at 2 kx (b) and 10 kx (c).

The sound adsorption coefficient of PF/NRL was measured from impedance tube test on 40% 30% and 20% of binder. The results showed in Figure 2. Since the sound absorption coefficient is dependent on the sound frequency. Therefore, the absorption coefficient frequency Hz curves were plotted by the mean values of samples for each binder percentage group. From the results, the NF/NRL0% shows the different results compared to others; that is, cover up mid-low (1500 Hz) and high (4500 Hz) frequency. It is indicated that at 1500 Hz and above 4500 Hz frequency, the sound absorption coefficient of the NF/NRL0% nearly reaches 0.8. Even the PF without binder showed a good coefficient value but the frequency was still below 1000Hz when compared with binding with binder. The coefficient values of PF/NRL20%, PF/NRL30% and PF/NRL40% were slightly increased, nearly 1.0 at lower 1000Hz. amount the binder content, the PF/NRL20% showed the highest absorption coefficient value at 0.98 due to the high porosity of PF/NRL20%. This result suggested the pineapple leaf fiber bound with natural rubber would be suitable for using in low sound frequency application.

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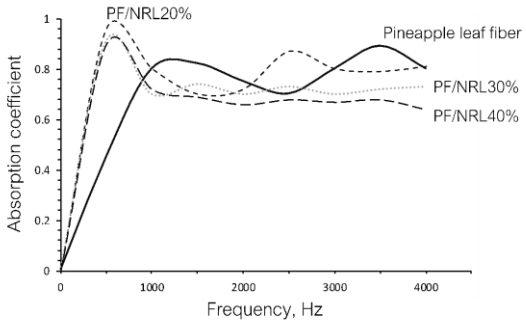

Figure 4. Absorption coefficient as a function of frequency of PF with and without NRL binder.

4. Conclusion

In this work, the pineapple leaf fiber bonded with natural rubber latex was successfully prepared by vary amount of natural rubber latex which acted as a binder**.** The sound absorption coefficient of the samples was investigated**.** The results indicated that the density and porosity of PF/NRL were playing an important role to improve the sound absorption**.** The PF/NRL20% showed the highest absorption coefficient value due the effect of density and porosity, while the absorption peak was slightly moved toward lower frequencies. It was the preliminary founding of sound isolation of pineapple leaf fiber bonded with natural rubber. More research should be done for preparing sound absorption material at low frequency in next work.

Acknowledgement

The authors are grateful to Rajamangala University of Technology Rattanakosin for supporting grant number C11/2564 to publish this work.

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Motorcycle Speed Calculation while Driving with an Animation from a Smartphone

Wutthikorn Malikong[1], Vachira Chaychumdee[1], Pannaporn Hirannawakun[1], Narong Sangwaranatee[1], Nisakorn Sangwaranatee[1], Piyawat Mankhong[2], Nuttapong Wattanasiripong [3*]

[1]Faculty of Science and Technology, Suan Sunandha Rajabhat University, U-thong Nok Road, Dusit, Bangkok 10300, Thailand
[2]Chemistry and Physics Subdivision, Central Police Forensic Science Division, Royal Thai Police
[3]Applied Mathematics, Faculty of Science and Technology, Valaya Alongkorn Rajabhat University
***Corresponding author e-mail: Nuttapong@vru.ac.th**

Received: *9 March 2022* / **Revised:** *7 April 2022* / **Accepted:** *5 October 2022*

Abstract

This research adapts basic technologies to develop a method for determining motorcycle velocity that may be used as evidence against those who exceed the speed limit in areas lacking speed detection devices. The speed was measured on Samsen roads in Bangkok, at Google Earth locations (1346'33.51"N 10030'25.96"E). The speeds of 40, 60, and 80 kilometers per hour were measured and recorded using two smartphones and placed in points A and B in two reference locations. The videos were then converted to image files to count the number of frames and time traveled on the motorcycle and estimate the movement speed. The measurement results showed that the values were not significantly different from those obtained from google earth at the significance level of 0.05. The data was analyzed by excel (t-Test: Two-Sample Assuming Equal Variances).

Keywords: Frame rate, Motorcycle, Velocity, Speed calculation

1. Introduction

Motorcycles are now popular vehicles in Thailand. The popularity of motorcycles is due to several factors such as convenience, speed and agility, the ability to reach the destination of the trip, the amount of fuel used, and maintenance costs lower than a car. Motorcycles are considered to be the first personal vehicles. However, the use of a motorcycle causes a danger from the user group, namely the risk of injury and death in a road accident.

From the Department of Land Transport statistics in 2019, comparing to the proportion of deaths from road accidents around the world, the highest percentage of fatalities in Thailand were caused by motor vehicle accidents (29%), followed by motorcycle accidents (28%), cyclists, and pedestrians (26%), and other road users (17%). Road accident deaths accounted for the highest number of fatalities caused by motorcycles, (74.40%), motor vehicle accidents, (12.30%), pedestrians (7.60%), cyclists (3.20%), and other road users (2.30%). It was clear that most fatalities were caused by road accidents in Thailand, with over 75 per cent of the population. As a result, Thailand is currently known for having the highest mortality rates globally when it comes to road accidents.

Street accidents are a major social problem that causes the loss of life and property. At present, the situation of traffic and transportation accidents has an increasing trend. According to road accident statistics from the Bureau of Safety Administration, Department of Highways, 73% of accidents on highways were presumed to be driving faster than the specified rate. The driving speed exceeding the legal limit is a common problem in Thailand, which is caused by improperly setting speed limits for the type of road use and land use on both sides of the road including a lack of strict enforcement of laws. The clarity of the enforcement of speed laws in Thailand is low compared to the enforcement of other road safety laws by the ineffective enforcement of speed control laws in Thailand caused by a shortage of law enforcement personnel and tools. In the past, autonomous speed detection technology systems are widely installed and operated on highways in Thailand. The agency responsible for road safety has recommended speed enforcement measures and implemented an automatic speed detection system (Kornprasert, 2017).

Statistics of the Royal Thai Police in 2019, when receiving reports of road accidents, found that the most accident-prone vehicles were: 1)

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motorcycles, 2) sedans, and 3) pickup trucks, respectively. There are many factors of accidents which can be divided into four groups: 1) the cause of the accident caused by the person, 2) the cause caused by the equipment, 3) the cause caused by the environment; and 4) the cause of the traffic light/sign, which is considered from the cases caused by the person (driver) from a road traffic accident reported in the year 2019, classified by vehicle type.

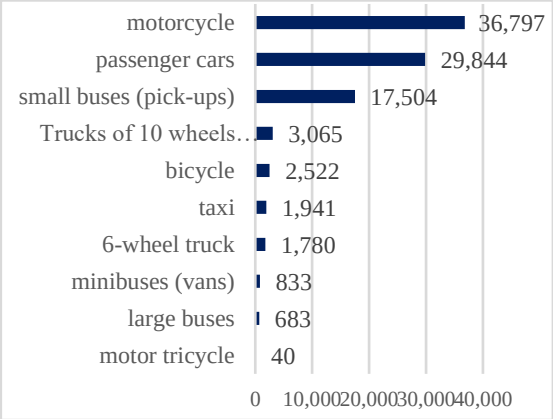

Figure 1. Statistics for car accident victims nationwide (Thai RSC, 2019).

The cause of motorcycle accidents is caused by 94% of drivers, with 54 percent of motorcyclists and 40 percent of motorcyclists being caused by motorcyclists. Causes of motorcycle accidents included: 52.30% of perception failure, 20.80% of decision failure, and offenses. Reaction failure was 18.8 percent, which was also found that 26.20% of all accidents caused by driver attention failure, and 40.30% of all accidents occur resulting from the decision to faulty traffic strategy. Over 85 percent of motorcycle accidents are caused by riders who have not been trained to ride safely but instead, learn how to ride a motorcycle from family, friends, or self-learning.

Strict enforcement of traffic laws is one of the ways that can lead to a significant reduction in motorcycling accidents. In which, imposing penalties on those who violate traffic laws, such as speeding beyond the legal limits. Arresting an act that violates the traffic law with credible evidence leads to punishment by the penalties prescribed by law. The researcher considers the importance of such problems. Therefore, it is necessary to study the speed of motorcycle driving on public roads. Speed, distance, and time detected from video recorded data were taken from the first to the second position. They can be used to determine the motorcycle speed, which may be applied for proving traffic offenses or as part of a traffic trial that can occur.

Rad, Dehghani and Karim (2010) studied a found that video processing was used for traffic surveillance, analysis, and monitoring of traffic conditions in cities and districts. The purpose was to offer other approaches to vehicle speed estimation. It collected images of traffic captured by still cameras mounted on expressways. The cameras were calibrated according to the geometric equations directly supported by the reference. Calibration of the camera for precise readings was achievable, but determining speed accurately was challenging. The designed system can be propagated to other traffic-related applications. The speed error detected was 7 kilometers per hour. The experiments were performed with different resolutions and different video sequences.

Shukla and Patel (2013) studied a new method of estimating the speed of ground vehicles, which was an automatic estimation of the vehicle's speed from a video sequence obtained from a permanently installed surveillance camera. The car movement was detected, which was followed by the different scenes using Lucas-Kanade algorithm distance travel by the vehicle. It was calculated using the center moves over the screens and the estimated vehicle speed. The speed of the vehicle was determined from different positions on the screens.

Kim, Oh, Choi and Park (2018). studied a method for estimating the speed of a moving vehicle with side camera images. A sufficient number of reference points of velocity vectors was a specified vehicle using various scene images, which involved two main steps. Firstly, there were enough points from the selected cars. These points must follow closely on at least two successive video scenes. Secondly, the tracked point displacement vectors and usage times were used. The velocity vectors of those points were calculated. The calculated velocity vectors were defined in the video image coordinate system. The displacement vectors were measured by pixel means. Then the magnitude of the vectors calculated in the image space were converted to the object area to determine the absolute value of these sizes. This transformation required an image to oppose the area data in mathematical terms achieved by the calibration mean and the orientation parameters of the video scene image. This research presented a solution to the problem of using side camera images mentioned.

Han (2016) studied different traffic accident types, including car crashes, pedestrian collisions, and hit-and-run accidents. In this paper, overspeeding was one of the main areas of traffic accident analysis. Therefore, analysis of vehicle speed during an accident was essential. The current

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article proposed a vehicle speed estimation (VSEM) method using a virtual plane and a reference line like a forensic science video. The reliability of VSEM was verified by comparing the results obtained from the Vehicle Speed Estimation (VSEM) method with the video of the test drive of the vehicle being driven with the Global Positioning System (GPS) based on the Vbox speed principle.

Doğan, Temiz and Külür (2010) found the use of original video footage (footage) of car cameras. The vehicle speed could be assessed directly from the recorded image without the need to locate the specific physical characteristics of the vehicle displayed in the recorded instrument. Moving car speed was estimated to analyze the actual results. The speed estimation cross-ratio was compared with the results of the original video analysis method (footage).

Chantruthai, Pansrinual and Jinwan (2017). found the speed of vehicles on highways in the community outside the city. Motorcycles are the vehicles in the community, followed by passenger cars and pickup trucks. The moving vehicle data in the community indicated that the passenger car speed exceeded the legal limit both in the area outside the community and in the community areas. The truck's travel speed would progressively decrease below the speed limit outside the village, however it was discovered that the return speed soared over the legal limit when entering the community.

Saisuksaard and Kronprasert (2018) found that the efficiency of using automatic speed cameras found that the speed control measures with automatic speed cameras caused the driver to use a significantly reduced driving speed. After installing an automatic speed detection system, personal vehicle speeds decreased by 7.7 percent. The bus used speed decreased by 8.8 percent. The psychological factors of drivers affecting speed behavior found that the cognitive factor controlling behavior had a high effect. Therefore, the best speed using the law enforcement measures based on speed control with automatic speed cameras could reduce the opportunity for drivers to exhibit more speed behaviors. As a result, the driver intends to reduce the speed of driving.

Based on the above research works, this article aims to propose a technique for the motorcycle speed calculation from a smartphone video file. This technique can potentially address motorcycle accidents in Thailand.

Video clip showing today's smartphones features in recording high quality video clips with sharper and higher frame rate from previous version that affects the accuracy of the vehicle speed calculation and using the Google Earth odometer to help find distance traveled by the car. Therefore, the researcher has initiated an idea that the aforementioned data can be used to calculate the speed of the car traveling on the road leading to its application as a tool to detect posed limit vehicles.

2. Objectives

1) To calculate the speed of a motorcycle using a video clip from a smartphone and Google Earth program.

2) To propose guidelines for finding evidence to punish those who drive faster than provided in the law.

3. Materials and Methods

3.1 Methodology

This research is a simulation of driving a motorcycle on a straight-line road. The road surface is Alfred on the daytime test, where the test in a place where there is a clear reference position on the Google Earth program (13°46'33.51"N 100°30'25.96"E) by using 1 test motorcycle, a 2017 Yamaha GT125 CC. Reference points A and B are records of the vehicle's movement with a video camera of a smartphone model SAMSUNG Galaxy A30 at the first and second position to use the data from the video file to calculate the time spent on the motorcycle in motion. Two methods were used to measure distances in this experiment: 1) measure the distance from Google Earth, and 2) measure distance with a standard distance meter (tape).

3.2 Variable studied

Independent Variable: This includes measuring the distance traveled by car in 2 ways: 1) measure distance from Google Earth and 2) measure distance with a standard distance measuring tool (tape measure).

3.3 Control variable

Control variable including motorcycles: The road environment in the experiment was the straight road. Paved road surface, dry road surface, day time trial. The researcher selected Samsen Road in front of St. Gabriel's School, which meets the definition of the control interpreter. The experiment was conducted during the period from 10.00 am to 12.00 pm because the traffic was not busy in which the experiments according to the experimental plan can be controlled effectively.

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3.4 Experimental method

This research simulates riding a motorcycle in a straightway through two reference points, A and B, at speeds of 40 km hr^{-1}, 60 km hr^{-1}, and 80 km hr^{-1}.

1) The measurement distance; Before measuring the distance, it is necessary to survey the area to be measured to know the exact route to be measured. There are two ways to follow. measure the distance traveled by car: 1) measure the distance from Google Earth and 2) measure the distance with a standard distance measuring tool (a tape measure), details as

1.1) The steps to measure distance with the Google Earth program are as follows.

(1) Launch the Google Earth program that will be used to analyze distances.

(2) Find an area to experiment. The measurement area is Samsen Road, in front of St. Gabriel's School.

(3) Mark reference point A to reference point B

1.2) Procedure for measuring distances with a tape measure as follows.

(1) Field the area for measuring the distances

(2) Mark the reference point.

(3) Measure the distance of reference A to reference B.

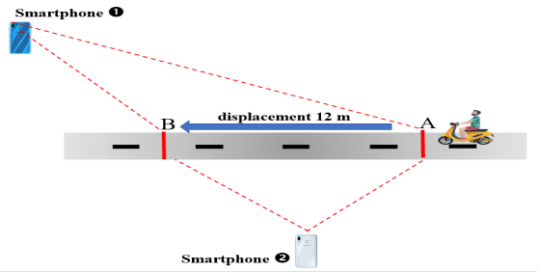

Figure 2. Determining the A and B positions for the experiment.

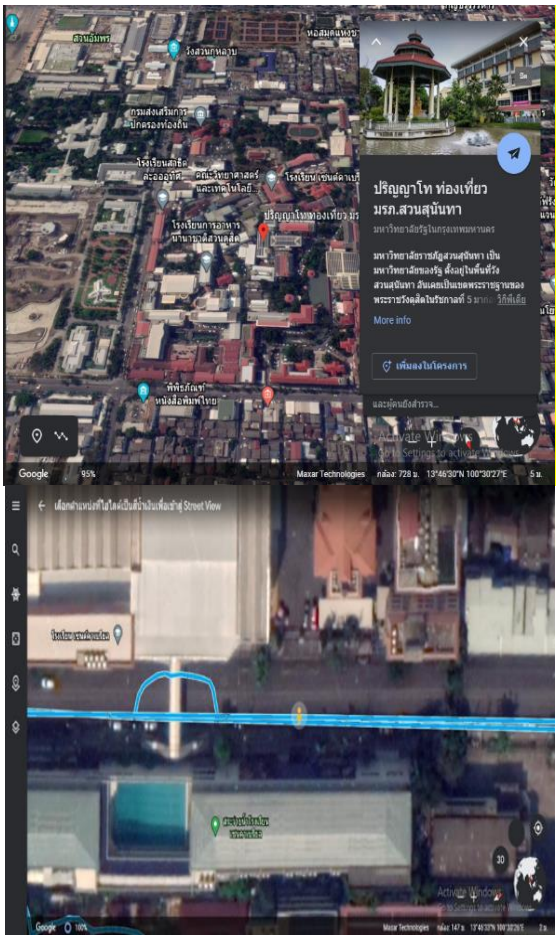

Figure 3. Samsen Road in front of St. Gabriel's School to set distance.

2) A smartphone was utilized to record the motorcycle's movement, with two identical smartphones being used for the recording. The motorcycle was then ridden at speeds of 40 km hr^{-1}, 60 km hr^{-1}, and 80 km hr^{-1} past reference location A to position B. At each speed level, the experiment was performed 30 times.

2.1) Location 1 was taken from a motorcycle's height (1st smartphone camera). Arrange for the installation of a video camera on the bridge. Adjust the camera's direction in accordance with the motorcycle's movement. During the experiment, the motorcycle was driven toward the camera.

2.2) Location 2 captures photographs perpendicular to the motion of the motorcycle (2nd smartphone camera). Install a surveillance camera on the sidewalk. Set the smartphone camera's direction perpendicular to the motorcycle's movement. The vehicles would move through the camera from right to left.

3) Converting video files to picture files.

3.1) A video file was created when a smartphone camera captures footage of moving cars.

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The vehicles that traveled from point A to point B were counted and recorded. The video to jpg converter was used to convert the video files to photos.

3.2) Calculate the travel time required to verify the frame rate of the recorded video file (Frame rate) by right-clicking the video file and selecting the properties menu. The frame rate of the video file showed in the details menu. It was expressed as the number of images recorded in 1 minute, for example, 30 frames/second, meaning that the video was recorded at a rate of 30 frames/second.

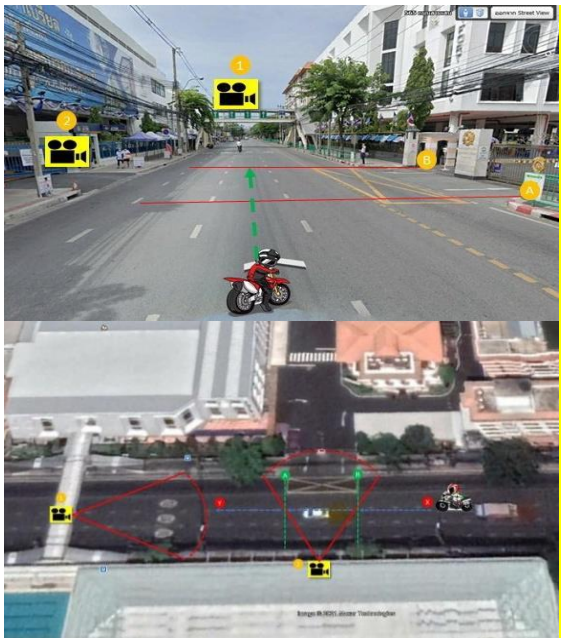

Figure 4. The location of the camera used to record and the direction of the recording of a motorcycle test ride, where 1, 2 were the positions of the camera. A, B were the reference points for measuring distances determined by the researcher.

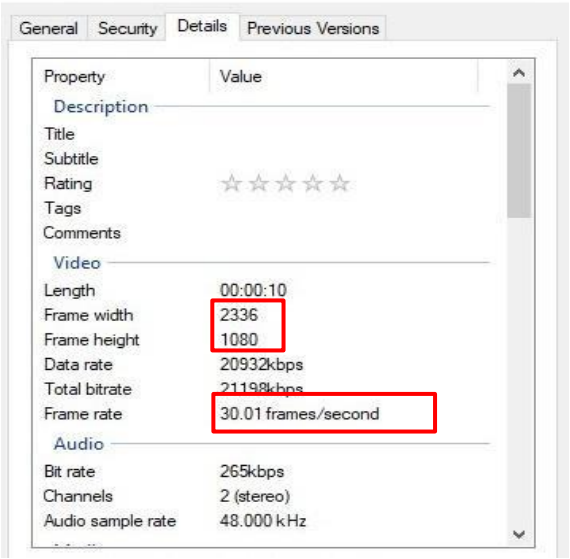

Figure 5. Find the frame rate with a free video to jpg converter.

4) Calculate the duration by calculating the number of frames required for the automobile to go from reference A to reference B using the formula below (1), where t is the amount of time the vehicle travels (seconds) between reference A and reference B, which is the frame rate of the video file.

$$t = \frac{n}{f} \quad (1)$$

Motorcycle speed calculation can be found from equation (2), which is given by

$$v = \frac{s}{t} \quad (2)$$

Where v is the average speed (meters per second) of the total distance with the traveling time t.

4. Results and Discussion

A tested motorcycle was at speeds of 40, 60, and 80 kilometers per hour. The time was obtained from video files from the smartphone camera of driving the motorcycle on a straight-line road. The road surface was Alfred. It was the daytime test on Samsen Road, from which the reference position on the Google Earth program was 13°46'33.51"N 100°30'25.96"E using 1 test motorcycle, a 2017 Yamaha GT125 CC. In a test ride moving through reference A and B was a record of the movement of an experimental vehicle with a video camera of a smartphone model SAMSUNG Galaxy A30 at the first and second positions to use the data from the video file to calculate the time spent on the motorcycle in motion. Two distance measurement

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methods were applied in this experiment: 1) measuring distances with Google Earth and 2) measuring distances with a standard distance meter (tape). The time and distance data obtained from the experiment used to calculate the speed of the vehicle test each speed level 30 times.

Table 1. The calculating speed at 40, 60, and 80 km hr^{-1} and counting the number of frames.

Speed (km hr^{-1})	Camera	Perpendicular angle (m)	
		Google Earth	Measurement
40	1	12.32	12.54
	2	8.28	8.23
60	1	12.32	12.56
	2	8.28	8.23
80	1	12.32	12.25
	2	8.28	8.23

However, according to the results, motorcycle speed calculation using data from mobile phones and distances measured by the Google Earth program was considered high accuracy, with the maximum discrepancy of 7.93%, which was in relation to the findings of Rad et al. (2010). This method would be beneficial for determining traffic violations, especially with vehicles driving over the speed limits; this method would be a good evidence source for offenders. For instance, if the legal speed limit is not more than 80 Km/h, and the actual speed of a motorcycle is 90 Km/h. Regarding the calculation, there was approximately 7.93% data discrepancy, resulting in the calculator displaying the data in the speed range of 82.07- 97.93 Km/h. From this, it could be proven that the previous vehicle exceeded the maximum speed limits. On the other hand, if an actual vehicle speed was at 85 Km/h, the speed calculation would range from 78.07-92.93 Km/h; this may cause future conflict if the driver's speed did not exceed the speed limits but got accused due to the false calculation.

Measuring distances by Google Earth, in some cases there may be inaccuracies of distance values depending on the accuracy of the user who determines the location from which the distance is measured. This can be caused by many factors, such as the sharpness of the image, current information, or even an angle that the program user chooses to use to determine the position.

5. Conclusions

This research calculated the speed of a motorcycle while driving with an animated smartphone. Speed test was on Samsen Road Bangkok, at speeds of 40, 60, and 80 km hr^{-1} at the Google Earth reference point (13°46'33.51"N 100°30'25.96"E), the test ride moves past the reference points A and B, recording the movement of the motorcycle with a camera. The 1st smartphone(camera) was firstly installed on the overpass position, where the second one was installed on the pavement perpendicular to the movement of the motorcycle. Data from video files were converted to image files to count the number of frames. Traveling time and distance of a motorcycle were used to calculate the speed of movement. It was assumed that the measured values were not different from those obtained from google earth when analyzing data analysis in excel using t-Test: Two samples were assumed to have equal variances. The result of the obtained speed is agreed with the hypothesis. It means that the values obtained from the measurements were not significantly different from those obtained from google earth at a significance level of 0.05.

6. Suggestions

This research presents the calculation of the speed of a motorcycle while driving with animation from a smartphone, which can apply to punish offenders in areas without speed detection. To prevent driving a motorcycle speed exceeding the legal limit will result in a decreased accident rate. This method can be applied to determine traffic violations with motorcycle driving speed exceeding the legal limit. This computational principle may be used as evidence for the arrest of the offender.

Acknowledgements

I would like to express my sincere thanks to the Faculty of Science and Technology, Suan Sunandha Rajabhat University, Faculty of Science and Technology, Valaya Alongkorn Rajabhat University and Central Police Forensic Science Division, Royal Thai Police for invaluable help throughout this research.

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Subsurface Integrity Assessments of a Proposed Plaza Building at Oniru Lekki, Lagos, South-western Nigeria, Using Geoelectrical and Geotechnical Methods of Investigations

Adegbite, Joseph Taye[1]*, Adeoti Lukumon[1], Johnson, Temitayo Olatilewa[1], Anukwu, Geraldine Chibuzor[1], Adeleke Taofik[1]

[1]Department of Geosciences, University of Lagos, Akoka, Lagos, Nigeria

***Corresponding author e-mail: Joseph.adegbite74@gmail.com, Joseph.adegbite@yahoo.com**

Received: *16 February 2022/* **Revised:** *7 October 2022/* **Accepted:** *19 December 2022*

Abstract

Subsurface integrity assessment for building foundation using geophysical and geotechnical methods was carried out at Oniru, Eti- Osa, Lagos State. A total of twenty-five Vertical Electrical Sounding (VES), five 2-D Resistivity Imaging Survey traverses and two borings with Standard Penetration Test (SPT) were carried out at the study site. The acquired data were processed quantitatively using partial curve matching and computer iteration technique to generate the geoelectric sections, the 2-D model and the SPT logs. The VES results revealed five to six geologic units corresponding to topsoil, clayey sand, sandy clay, clay/peat, and sand while the 2-D resistivity structure corresponds with the VES result. The borehole log reveals sand layers having an N-value of 22-30 which is indicative of medium-dense, brown (medium to fine) sand with occasional gravels from depth 1.50 - 7.50m. The borehole log information correlates well with the VES/2-D result. For the sand layer which connotes dense, grey (medium to fine) sand with occasional gravels from a depth 7.50 – 15.75m with N-value ranging from 26–30m. The study analysis shows that the proposed building could be placed on the dense sand at depth 7.50 – 15.75m, this, however, should depend on the proposed load, length, and breadth of the proposed building. This study has provided useful information about the subsurface condition for engineering structure and zone suitable for the proposed foundation.

Keywords: Assessment, Building-foundation, Integrity, Geotechnical, Subsurface

1. Introduction

These days, the statistics of engineering structures failures have increased geometrically, several probable reasons speculated to be responsible for this ugly incident highlighted by the engineering community. These include inadequate supervision, poor construction materials, non-compliance to specification and host of others. However, one critical point that has always not been given due attention is the availability of adequate information on the nature of subsurface conditions prior to construction exercise. Foundation design depends on the characteristics of both the structures and the subsurface materials. Therefore, the competence, strength and load capacity of the soil supporting the super structure becomes an extremely important issue for the integrity and durability of the engineering structure (Akintorinwa & Abiola, 2011).

Globally, in many coastline terrains, the near surface soil is composed of expansive clay, a material that behaves differently compared to sandy soil which does not expand when it gets wet. Instead, the pore spaces in between the grains are filled by water. Because of this, the soil volume does not change and there is little movement of structures supported by the soil when the soil moisture conditions alternate between wet and dry. Although, since every engineering structure is seated on geological earth materials, it is imperative to conduct a lithological investigation of the subsurface materials of the proposed site to ascertain the strength and fitness of the host materials (Olorunfemi & Mesida, 1987; Oyedele & Okoh, 2011).

Potential of geophysical prospecting in engineering investigations is yet to be fully maximized; this is because of its merits of enabling information to be obtained for large volumes of ground that cannot be investigated by direct methods because of the costs involved. Several geophysical methods have been deployed for both pre- and post-construction investigation, geophysical investigation including gravity, electrical resistivity, electromagnetic (EM), and

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seismic refraction methods have been employed in different areas of applied geophysics for about a century, particularly for shallow and near-surface investigations (Aizebeokhai, 2010; Loke, 2001; McDowell et al., 2002; Roth, Mackey, Mackey, & Nyquist, 2002). The use of geoelectrical resistivity for investigating subsurface layered materials has its origin from the work of Conrad Schlumberger who conducted the first geoelectrical resistivity experiment in the fields of Normandy; and similar idea was developed by Frank Wenner in the United State of America. Ever since, geoelectrical resistivity surveying has greatly improved, and has become an important and useful tool in hydrogeological studies, mineral prospecting, and mining, as well as in environmental and engineering applications (Aizebeokhai, Olayinka, & Singh, 2010; Amidu & Olayinka, 2006; Ayolabi, Adeoti, Oshinlaja, Adeosun, & Idowu, 2009; Ayolabi, Enoh, & Folorunso, 2013; Coker, 2015; Ehibor & Akpokodje, 2019; Fajana, Olaseeni, Bamidele, & Olabode, 2016; Kunetz, 1966).

To have adequate knowledge of the subsoil behaviors of the individual materials, geotechnical investigation is necessary to ascertain the engineering properties of the subsoil materials that would have direct interaction with structures in a particular area. Standard penetration tests (SPT) and Cone penetration test (CPT) are some of the commonly applied techniques used for this purpose this is because they are accurate, fast, and economic methods by which foundation soils can be characterized in terms of stratigraphy, associated strength and deformation characteristics (Adebajo, 2005).

The study area, a commercial center primarily an event center, consists of a pre-existing bungalow building with a prospect of having a multi-story structure for events hosting and functions. Therefore, due to the coastal plain sediment deposits in the study area, there is a need to carry-out adequate pre-foundation investigation in which this study was used to address. Combined geophysical and geotechnical investigation methods were used to characterize the subsurface materials.

1.1 Study area and the geology

The study area is the Oniru Beach which lies within latitude N 06º 25' 19.7" to N 06º 25' 22.1'' and longitude E 003º 26' 33.2" to E 003º 26' 39.1" in Oniru Beach, Ozumba Mbadiwe Avenue, Eti-Osa, Lagos, State South-western Nigeria. It is bounded by the clean sea water and variety of restaurants (Jones & Hockey, 1964). The map of Lagos shows different Local Government Areas (Figure 1).

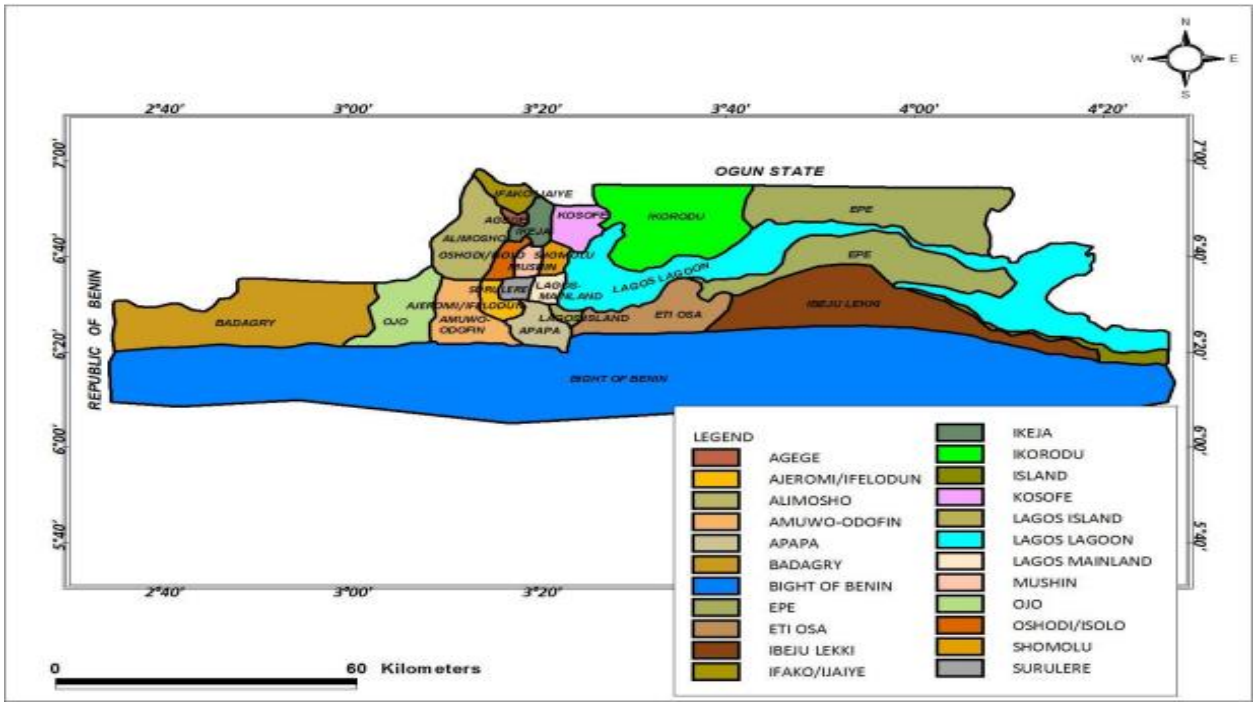

Figure 1. Map of Lagos showing Local Government Area (Afolabi, Oluwaji, & Fashola, 2017).

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The geology of the area in Lagos is mainly sedimentary, comprising tertiary and quaternary sediments. There is no basement outcrop within the state because its basement is several kilometers beneath the earth surface. Lagos states coastline zones with creeks and lagoons developed by barrier beaches and sand deposition lies on the longitude 3^0E and latitude 6^0N with alternate wet and dry seasons. Tertiary sediments are unconsolidated sandstones, grits with mudstone bands and sand with layers of clay. Quaternary sediments are recent deltaic sands, mangrove swamps and alluvium near the coast (Jones & Hockey, 1964). Figure 2 shows the base map of the survey site.

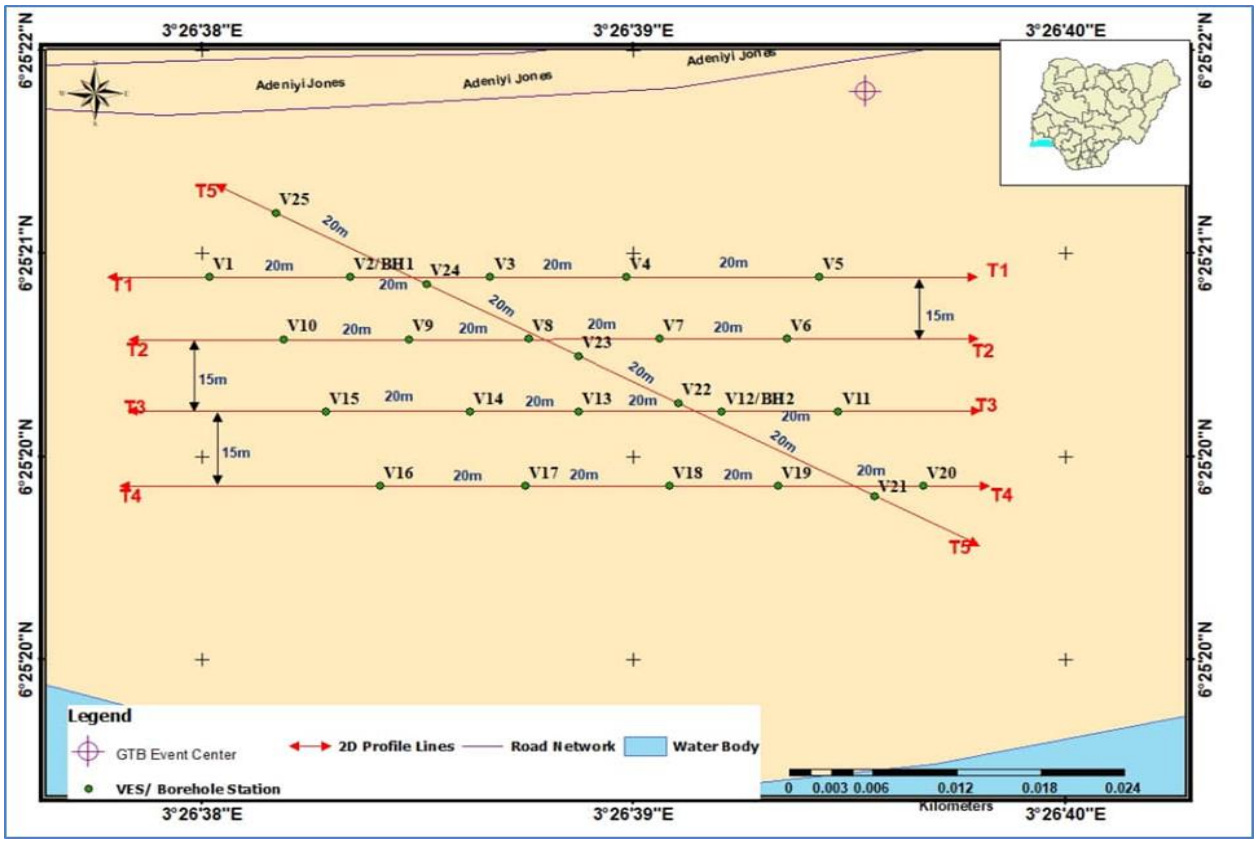

Figure 2. Base map of the survey site.

1.2 Basic theory of electrical resistivity

The basic theory of electrical resistivity for a conductive material described by one-dimensional body the relationship between the current and potential difference is defined by Ohms' law (Kearey, Brook, & Hill, 2002) in equation 1**:**

$$V = IR \quad (1)$$

Where the constant of proportionality, R, is known as the resistance and is measured in ohms where current **(**I**)** is in amps and voltage **(**V**)** is in volts (Kearey et al., 2002).

One of the most common arrays used in resistivity surveying is the Schlumberger array. The Schlumberger array is arranged with two current electrodes on the outside of the array, set apart by a distance at least five times the spacing between the two interior potential electrodes (Figure 3). The potential difference measurement is believed to lie at the mid span of the interior potential electrodes, at a depth approximately one half of the length between the exterior current electrodes.

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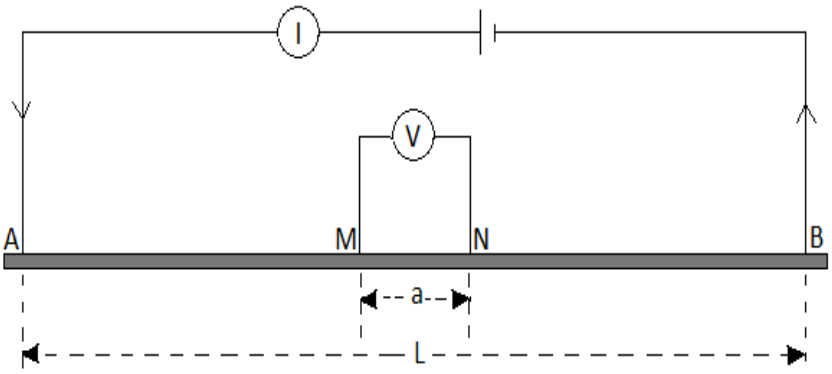

Figure 3. Schlumberger array (Milsom, 2003).

1.3 Basics of geotechnical investigation

The British Standards code of practice for site investigations, B.S 5930:1981 and the Methods of Testing Soils for Civil Engineering Purposes, B.S 1377:1975, have the generally acceptable methods used for boring, sampling, in-situ testing and describing soils. Boring in soil is mainly by the "Shell and Auger" or "Cable Percussion" method. This is based on the use of a variety of tools which, except for the auger, are alternatively raised and dropped to break up and recover the soil. Undisturbed samples of cohesive soils are taken with a 100 mm (approximate) internal diameter open tube sampler fitted with a cutting shoe. This consists of a split barrel thick-walled sampler (split spoon) of about 35mm internal diameter is driven 450mm into the soil by repeated blows from a trip hammer weighing 65Kg and falling through 760mm. The Standard Penetration Test Resistance, or "N_{SPT}"-value gives an empirical measure of the soil consistency and is also used to estimate the bearing capacity and compressibility of granular soils. The cutting shoe is often replaced with a solid cone for use in gravels (Sanchez-Salinero, Roesset, Shao, Stokoe, & Rix, 1987).

2. Materials and Methods

2.1 Data acquisition

A total of five (5) 2D traverses and wenty-five VES stations were acquired at differen points, as shown in Figure 2. The Wenner array electrode was used for the 2D resistivity imaging data acquisition with the length of spread of 200 m and sequences of electrode spacing at 10, 20, 30, 40, 50 m. While for the VES, the Schlumberger array was used, and the current electrode separation (AB) was varied out from a minimum of 2 to 200 m and the ge0detic system of coordinates was obtained using Garmin 12 GPS.

Two (2) boreholes were drilled within the study area as undisturbed samples at every location were taken at appropriate intervals using a specially designed 60.5 mm internal diameter U-Type sampler. The sampler was fitted with a cutter at the open end and a waste barrel at the other end. A round steel ball in the driving head of the sampler permits the escape of air and water as the sampler enters the tube. The diameter of the sample tube is 25 mm and lined with 60.5 mm plastic tube. The samples were trimmed to the desired length and usually 15 cm covered in a plastic tube. An identification label was attached. The number of blows required to drive the sample 15 cm into the ground was recorded. Sometimes, the regular U4 sampler is used to recover the undisturbed sample.

2.2 Data processing

The measured apparent resistivity VES data were processed both quantitatively and qualitatively. The quantitative interpretation of the depth sounding curves was carried out using the partial curve matching technique (Bhattacharya & Patra, 1968). To deduce the true resistivity distribution, a computer inversion software

WINRESIST was used. The result of the computer iteration shows the quantitative analysis to know the resistivity, thickness, and depth. This was presented in the form of a geoelectric section with the aid of AUTOCAD software. The 2D apparent resistivity data inversion was done using the DIPROFWIN program. The field data pseudo section and the 2D resistivity structure were produced after running the inversion of the raw data to filter out noise and the resistivity of each block was then calculated to produce an apparent resistivity pseudo section. The soil samples were taken to the engineering laboratory at the University of Lagos, where the soil analysis was carried out.

3. Results and Discussion

3.1 Geoelectric sections results

Along AA', figure 4 consists of five (5) VES stationed at 70, 90, 110, 130, and 150 m respectively. A total of five to six geoelectric layers which vary from layer one topsoil characterized by resistivity values and layer thickness ranging from (622.1 to 6356.9 Ohm-m; 0.6 to 0.8 m); second layer clay/ sandy clay/sand having resistivity values and thickness ranging from (104.7 to 746.9 Ohm-m; 1.2 to 2.6 m), and along with VES 1 to 3 the resistivity and thickness values ranges from (54.3 to 93.1 Ohm-m; 3.2 to 5.9 m); the third layer in VES 4 and 5, depicts clay with resistivity and thickness values ranging from (33.4 to 44.0 Ohm-m; 3.7 to 5.7 m); the fourth horizon beneath VES 1 is indicative of clay with resistivity value and thickness of (24.7 Ohm-m; 24.4 m), while the fourth geoelectric layer in VES 2 connotes sandy clay having resistivity and layer thickness value of (50.0 Ohm-m; 4.2 m). However, the fourth layer in VES 3 to 5 is diagnostic of sand with resistivity and thickness value ranging from (114.2 to 155.7 Ohm-m; 5.6 to 15.6 m); the fifth substratum layer in VES 1 is representative of sand having a resistivity value of (134.2 Ohm-m), but the layer thickness could not be determined because the current terminated within this zone. While the fifth layer in VES 2 represent clayey sand having resistivity and thickness value of (94.6 Ohm-m; 15.8 m). However, the fifth geoelectric layer in VES 3 to 5 signified clay with resistivity values ranging from (16.8 to 22.9 Ohm-m). The layer thickness in VES 3 is (23.3 m), but the layer thickness in VES 4 and 5 could not be determined due to current terminated within this region. The sixth geoelectric layer beneath VES 2 and 3 is symptomatic of sand having resistivity values ranging from 346.4 to 1007.8 Ohm-m, but their layer thickness could not be determined due to the current terminated within this zone. The sand in this zone represents a competent layer that can withstand engineering structures.

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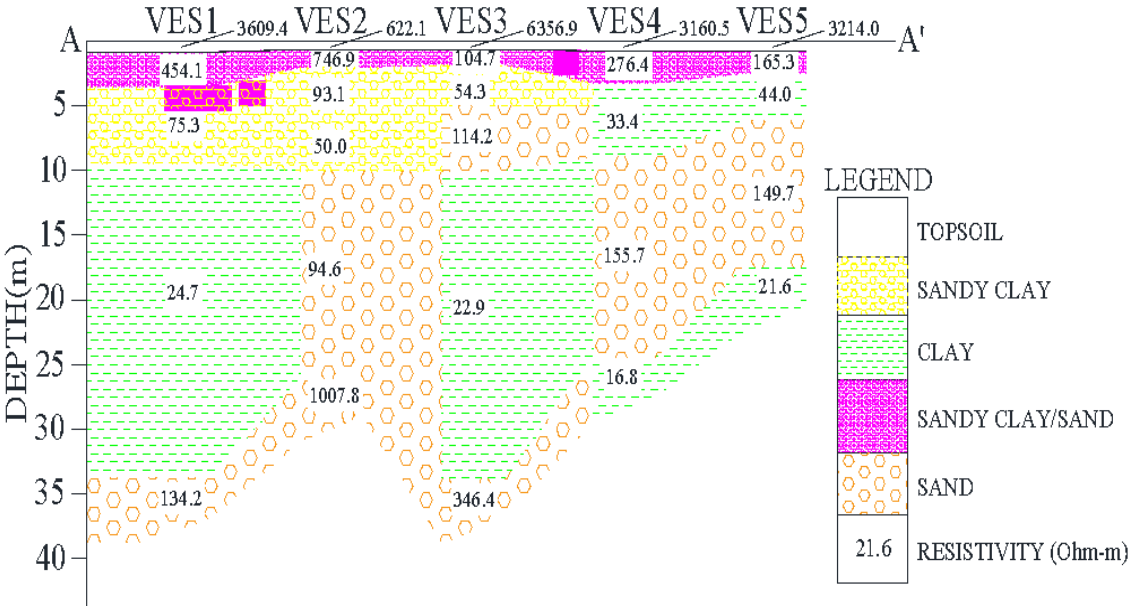

Figure 4. Geoelectric section for VES 1, 2, 3, 4 and 5.

Figure 5 along BB' consists of VES 6 to 10 stationed at 50, 70, 90, 110 and 130 m respectively. The section reveals five geoelectric layers which varied from; The topsoil having resistivity values and thickness ranging from (23.0 to 2971.7 Ohm-m; 0.4 to 0.7 m); The second identified layer in VES (6 and 8) denotes clay having resistivity and thickness values from (18.5 to 21.8 Ohm-m; 2.0 to 2.2 m), while the second layer in VES 7 revealed clay/peat with resistivity and thickness value of (8.3 Ohm-m; 2.3 m). However, the second layer in VES (9 and 10) represents sandy clay/sand with resistivity and layer thickness values of 145.1 to 1157.2 Ohm-m and 1.8 to 2.3 m respectively; The third geoelectric units along VES (6 to 8 and 10) denote sand having resistivity and layer thickness ranging from (120.3 to 284.1 Ohm-m; 4.3 to 5.5 m), while the third layer in VES 9 depicts clay with resistivity value of 43.5 Ohm-m and layer thickness of 3.8 m; The fourth horizon beneath VES 6, 8 and 10 is indicative of clay with resistivity and thickness values ranging from (12.8 to 35.0 Ohm-m; 12.0 to 48.6 m), while the fourth geoelectric layer in VES 7 and 9 connotes sand having resistivity and layer thickness values ranging from (244.2 to 467.4 Ohm-m; 12.0 to 13.0 m); The fifth substratum layer in VES 6, 8 and 10 is symptomatic of sand having resistivity values ranging from 108.8 to 246.8 Ohm-m, but their layer thickness could not be determined because the current terminated within this zone; the fifth layer in VES 7 and 9 is represent clay having resistivity values ranging 20.1 to 21.6 Ohm-m, but their layer thickness could not be determined due to current terminated within this region. The sand zone in the third geoelectric unit represents a competent layer that can withstand engineering structures.

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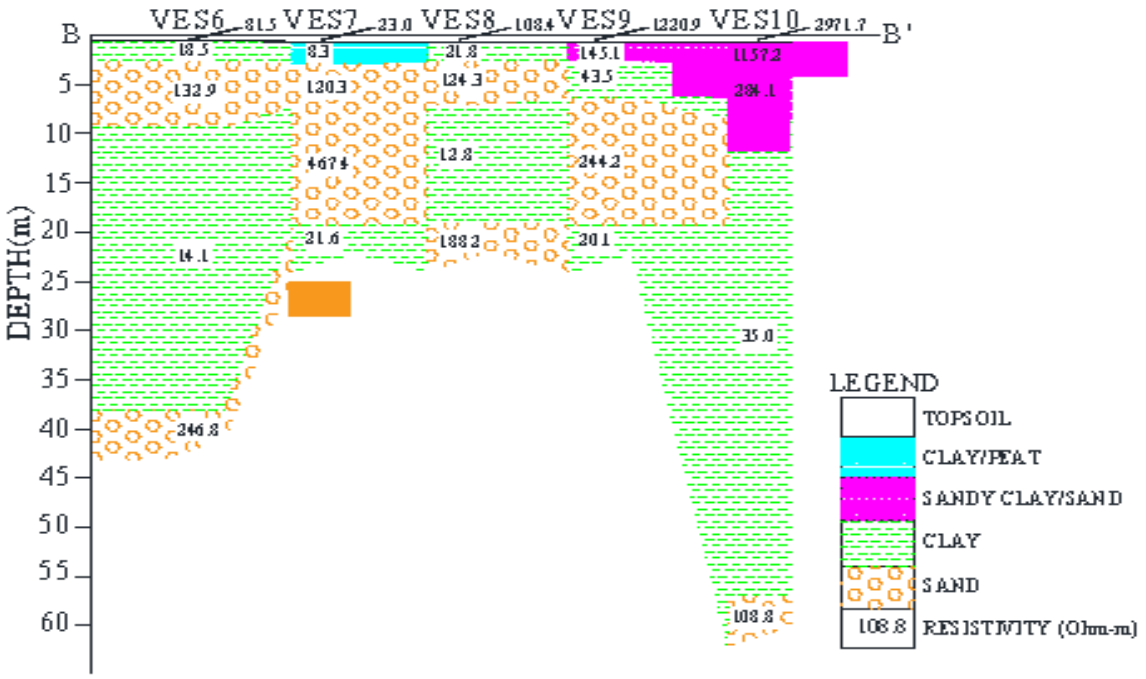

Figure 5. Geoelectric section for VES 6, 7, 8, 9 and 10.

Along section CC', in figure 6 consists of VES 11 to 15 stationed at 60, 80, 100, 120 and 140 m respectively along the 2-D traverse. The geoelectric section reveals four to six layers. The topsoil has resistivity and thickness values ranging from (37.7 to 2658.0 Ohm-m; 0.6 to 0.8 m); The second identified layer in VES 11, 12, 13, 14 and 15 represent sandy clay/sand, clay, and sand with resistivity and thickness values of (188.6 Ohm-m, 90.5 Ohm-m, 19.7 to 45.3 Ohm-m, 119.9 Ohm-m; 3.6 m, 1.4 m, 1.6 to 2.6 m, 3.5 m) respectively. The third geoelectric units denote clay, sandy clay, clayey sand and sand with resistivity and layer thickness values ranging from (13.1 to 46.3 Ohm-m, 60.3 Ohm-m, 83.7 Ohm-m, 200.3 to 251.3 Ohm-m; 2.6 to 26.5 m, 1.3 m, 5.1 m) respectively, except at VES 11, 14 and 15, where the layer thickness could not be determined because the current terminated within this zone. While the fifth layer in VES 13 is symptomatic of sand having a resistivity value of 410.0 Ohm-m but the layer thickness could not be determined due to current terminated within this region. The sixth geoelectric layer beneath VES 12 was diagnostic of sand having a resistivity value of 293.6 Ohm-m but the layer thickness could not be determined due to current terminated within this zone. The sand zones represent a competent layer that can withstand engineering structures while the clay, clayey sand and sandy clay signify an incompetent zone for mega engineering structures.

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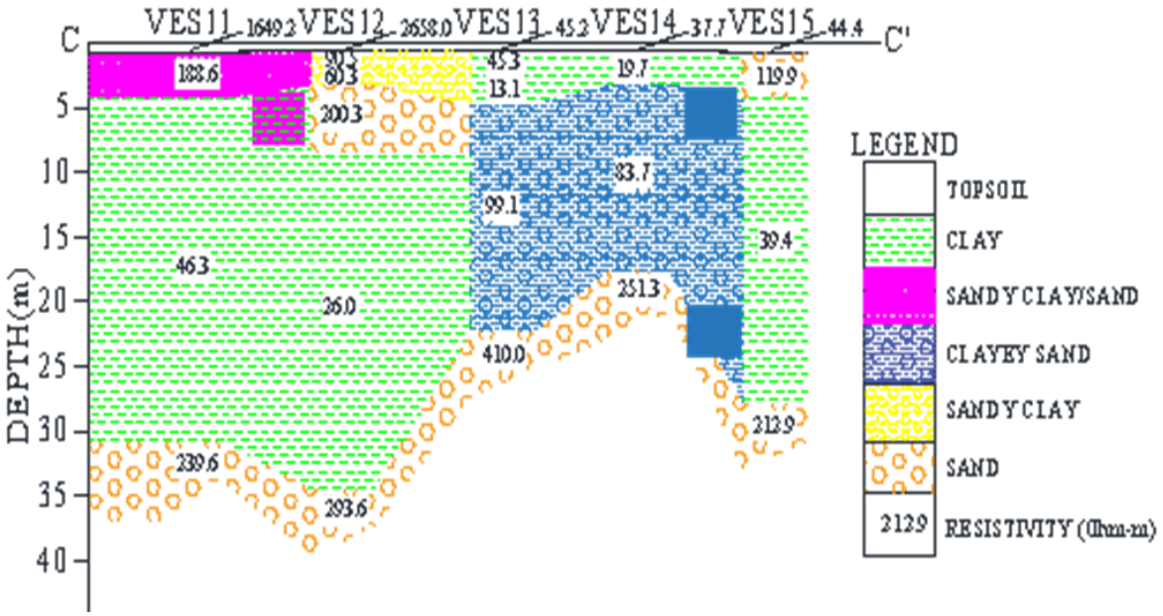

Figure 6. Geoelectric section for VES 11, 12, 13, 14 and 1

The geoelectric Section along DD' in Figure 7 consists of VES 16 to 20 stationed at 60, 80, 100, 120 and 140 m respectively. The section reveals four to five geoelectric layers which vary from topsoil, clay/peat, clay, clayey sand, sandy clay/sand, and sand. The topsoil is characterized by resistivity values and thickness ranging from (30.9 to 1661.7 Ohm-m; 0.6 to 0.8 m); The second identified layer represent sandy clay/sand having resistivity and thickness values ranging from (14.2 to 336.1 Ohm-m and layer thickness of 2.1 to 3.9 m); The third geoelectric units denote sandy clay, sand having resistivity and layer thickness values ranging from (59.9 to 82.5 Ohm-m, 187.8 Ohm-m; 3.1 to 7.7 m, 7.0 m) respectively. However, the third layer in VES 19 and 20 connotes clay/peat and sand with resistivity and thickness values of (9.8 Ohm-m, 84.3 Ohm-m; 5.7 m, 4.3 m) respectively; The fourth horizon layer is an indicative of clay with resistivity and thickness values ranging from (10.3 to 44.6 Ohm-m; 16.3 to 19.7 m), but the layer thickness in VES 18 could not be determined because the current terminated within this zone. While the fourth geoelectric layer in VES 17 and 19 connotes sand having resistivity and thickness values ranging from (113.6 to 473.3 Ohm-m; 8.0 m), but the layer thickness in VES 19 could not be determined because the current terminated within this region. The fifth substratum layer is a symptomatic of sand having resistivity values ranging from 158.4 to 188.4 Ohm-m, but their layer thickness could not be determined because the current terminated within this horizon. The sand zone represents a competent layer that can withstand engineering structures while the clay, clay/peat, clayey sand, and sandy clay connotes an incompetent zone because of the magnitude of the engineering structure that would be erected on the site.

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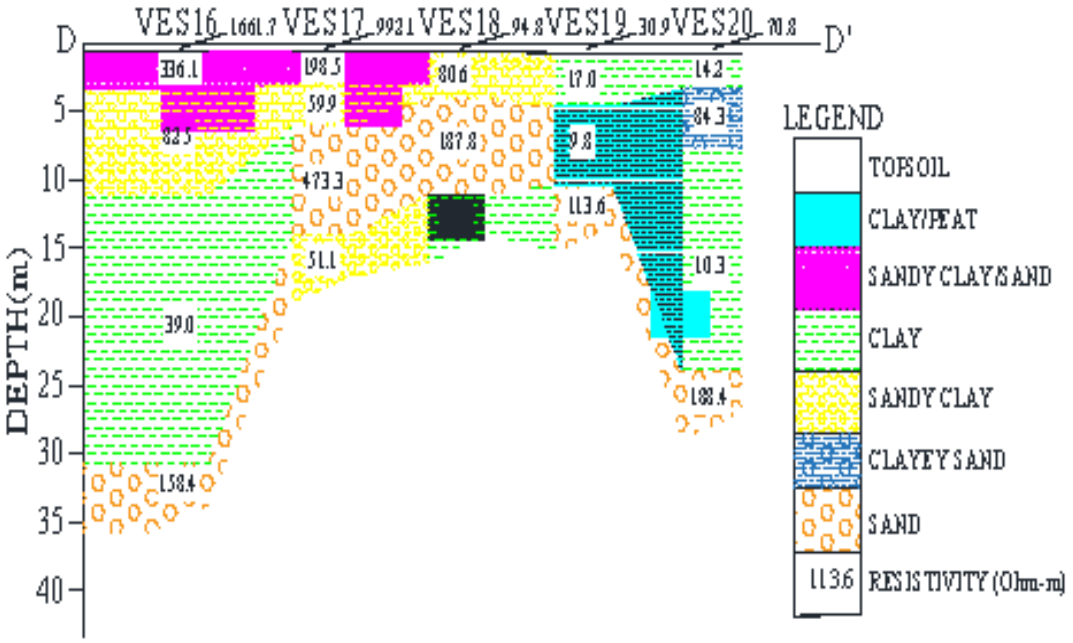

Figure 7. Geoelectric section for VES 16, 17, 18, 19 and 20.

The geoelectric section along EE' in figure 8 consists of VES 21 to 25 stationed at 60, 80, 100, 120 and 140 m respectively. The section reveals four geoelectric layers which varied from topsoil, clay, sandy clay, clayey sand, sandy clay/sand, and sand. The topsoil is characterized by resistivity values ranging from 37.9 to 2444.3 Ohm-m and layer thickness of 0.6 to 0.8 m; The second identified layer denote sandy clay/sand having resistivity values ranging from 108.8 to 339.9 Ohm-m and layer thickness of 1.7 to 4.4 m; The third geoelectric units represent sandy clay having resistivity and layer thickness values ranging from 52.6 to 58.7 Ohm-m and 7.0 to 12.5 m respectively. However, the third geoelectric layer in VES 25 is representative of clay with a resistivity value of 42.2 Ohm-m and layer thickness of 35.5m. The fourth stratum is symptomatic of sand having resistivity values ranging from 139.6 to 379.1 Ohm-m, but their layer thickness could not be determined because the current terminated within this zone. The zone with dense and medium dense sand represents a competent layer that can withstand mega engineering structures.

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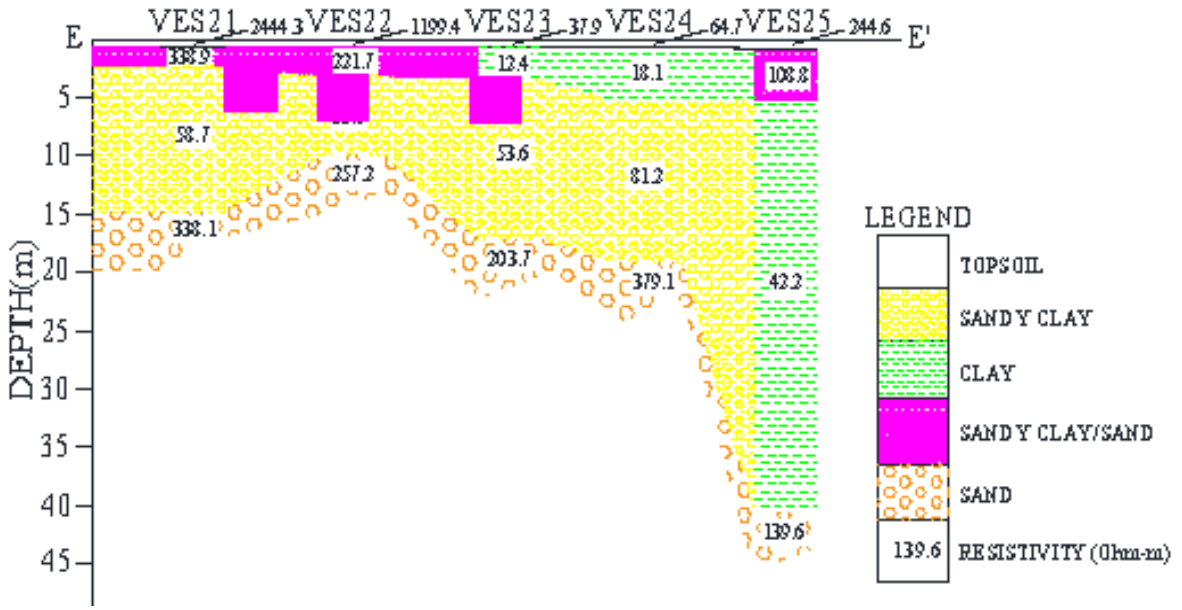

Figure 8. Geoelectric section for VES 21, 22, 23, 24 and 25.

3.2 2-D imaging results:

3.2.1 Traverse one

The total spread of 200 m with a depth of 50 m was probed with resistivity values ranging from 15 to 329 Ωm as shown in Figure 9. The borehole log one was along with a 2-D profile at a lateral distance of 90 m. At depth below 20 m is diagnostic of clay, clayey sand/sandy clay, and sand having a resistivity value ranging from 15 to 178 Ωm across the profile. The depth above 20 m to the subsurface depicts clay, clayey sand/sandy clay, and sand with resistivity in the range of 28 to 329 Ωm across the profile. The sand is distinctive at the depth of 25 to 50 m with a lateral distance of 95 to 170 m across the profile with resistivity values ranging from 178 to 329 Ωm. The sand zone represents a competent layer that can withstand engineering structures while the clay and clayey sand/sandy clay signified an incompetent layer for engineering structures.

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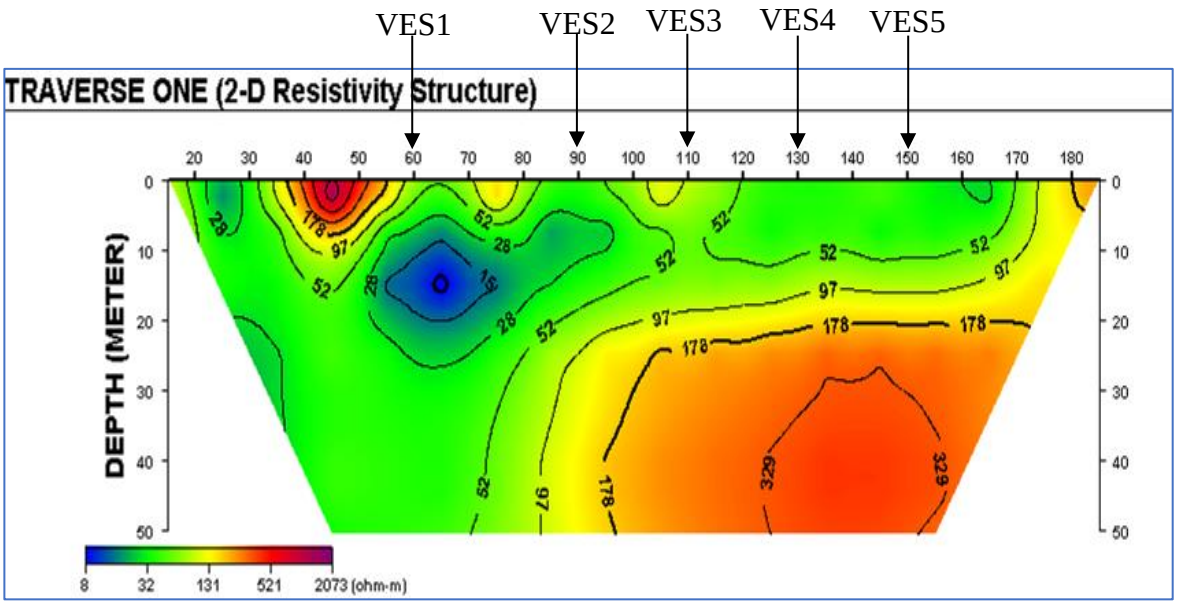

Figure 9. 2-D resistivity structure of traverse one

3.2.2 Traverse two

Traverse two has a profile length of 200 m and depth coverage of 50 m, with resistivity values ranging from 12 to 92 Ωm as shown in Figure 10. At depth below 20 m, clayey sand/sandy clay having resistivity values ranging from 29 to 92 Ωm across the spread was suspected. The depth above 20 m to the subsurface is an indicative of clay with resistivity in the range of 12 to 37 Ωm across the profile. The clay and clayey sand/sandy clay represent an incompetent layer for engineering structures.

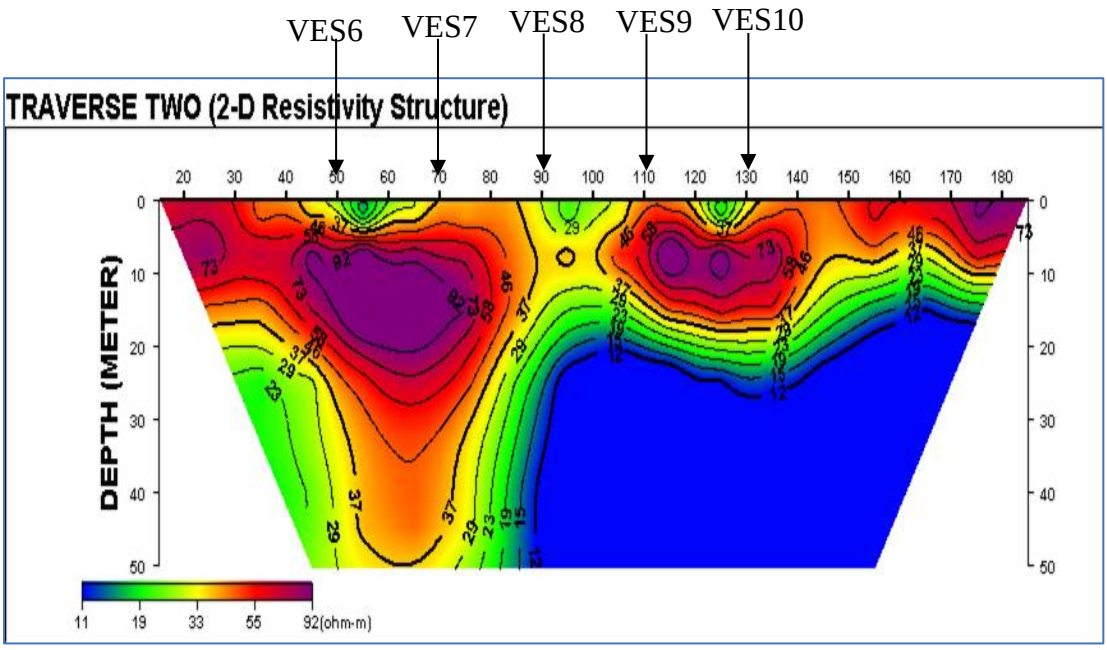

Figure 10. 2-D resistivity structure of traverse two.

3.2.3 Traverse three

This location has a total electrode spread of 200 m with 50 m depth of penetration. The resistivity values range from 8.5 to 238 Ωm as shown in Figure 11. The borehole log two was along with a 2-D profile at a lateral distance of 80 m. At depth below 20 m, clay/peat, clay, clayey sand/sandy clay, and sand having a resistivity value ranging from 8.5 to 148 Ωm across the profile was suspected. The depth above 20 m to the subsurface depicts clay, clay/peat, clayey sand/sandy clay, and sand with resistivity in the range of 8.5 to 238 Ωm across the profile. The sand is distinctive at the depth of 15 to 50 m with a lateral distance of 40 to 75 m across the profile with resistivity values ranging from 148 to 238 Ωm. The sand zone represents a competent layer that can withstand engineering structures while the clay, clay/peat, and clayey sand/sandy clay signified an incompetent layer for engineering structures.

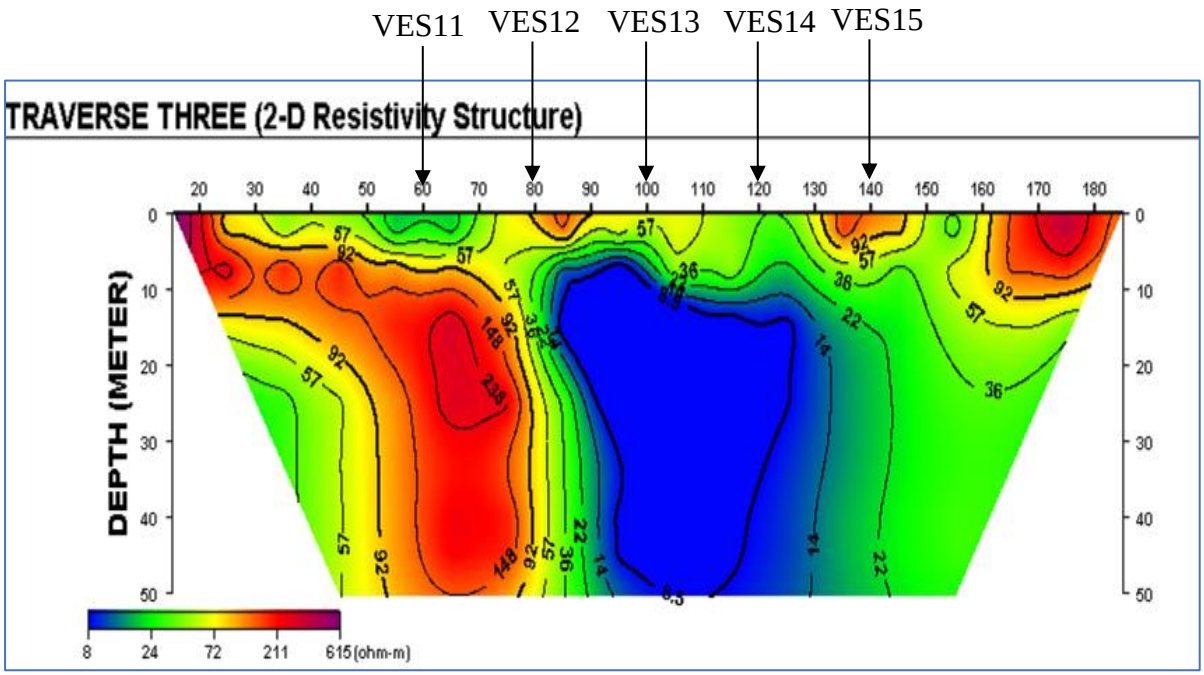

Figure 11. 2-D resistivity structure of traverse three.

3.2.4 Traverse four

The profile length of 200 m was occupied in this location with a 50 m depth of investigation. As shown in Figure 12, the resistivity values range from 3 to 72 Ωm, and at depth below 20 m is diagnostic of clay and clayey sand/sandy clay having a resistivity value ranging from 12 to 72 Ωm across the profile. The depth above 20 m to the subsurface connotes clay and clay/peat with resistivity in the range of 3 to 36 Ωm across the profile. The clay, clay/peat, and clayey sand/sandy clay are indicative of an incompetent layer for engineering structures.

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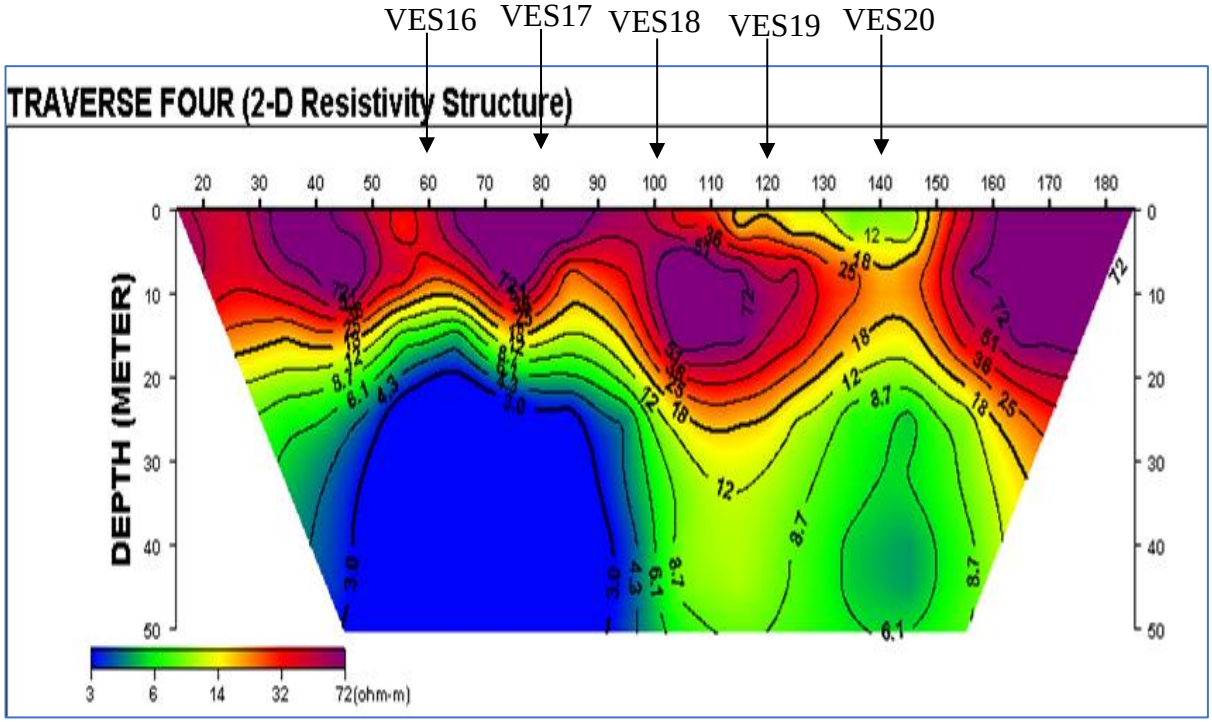

Figure 12. 2-D resistivity structure of traverse four.

1.6.5 Traverse five

The total spread of 200 m and a depth of 50 m was probed with resistivity values ranging from 20 to 985 Ωm as shown in Figure 13. At depth below 20 m revealed clay, clayey sand/sandy clay, and sand having a resistivity value ranging from 20 to 985 Ωm across the profile. The depth above 20 m to the subsurface is representative of clay and clayey sand/sandy clay with resistivity in the range of 20 to 61 Ωm across the profile. The sand is distinctive at the depth of 0 to 10 and 10 to 15 m with a lateral distance of 40 to 80 m and 130 to 160 m respectively across the profile with resistivity values ranging from 107 to 985 Ωm. The sand zone represents a competent layer that can withstand engineering structures while the clay and clayey sand/sandy clay signified an incompetent layer for engineering structure

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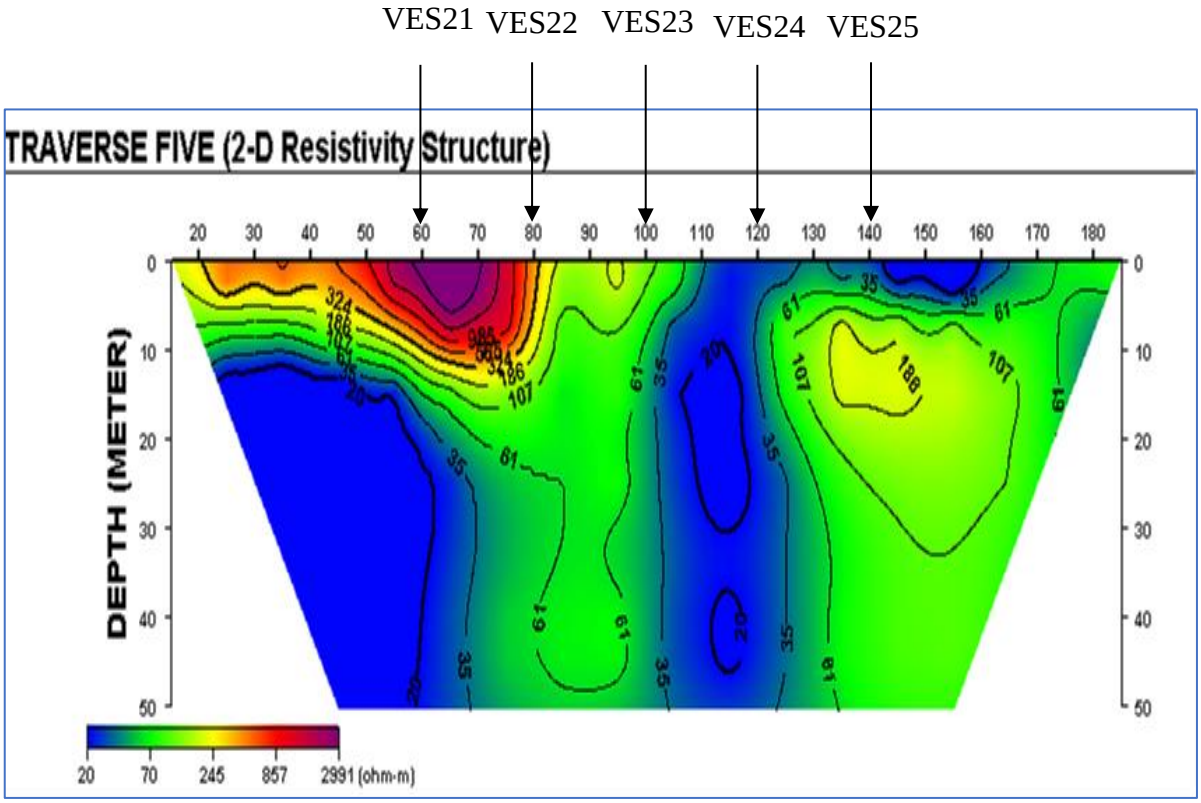

Figure 13. 2-D resistivity structure of traverse five.

3.3 Geotechnical results

The borehole logs obtained from the geotechnical analysis are displayed in Figures 14 (a and b) The first layer of the borehole strata which has no N-Value, revealed brown/grey medium to fine sand with occasional gravels from the ground level to a depth of 1.5 m in borehole (1 and 2). The second zone of the borehole logs has N-Value of 22 to 30 which is indicative of medium-dense, brown medium to fine sand with occasional gravels in the vicinity of the borehole (1 and 2) from a depth of 1.50 - 7.50 m. The third zone of the borehole logs revealed dense, grey medium to fine sand with occasional gravels from a depth of 7.50 – 15.75 m with N-Value ranging from 26 to 30.

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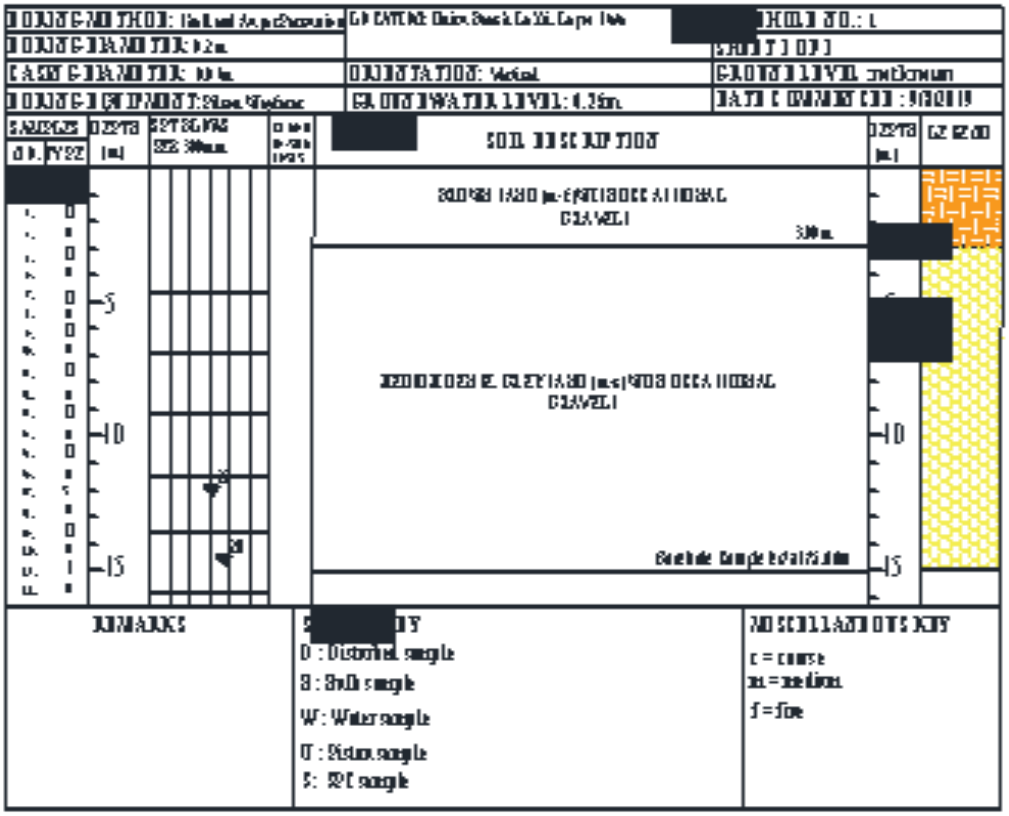

Figure 14a. Borehole log 1.

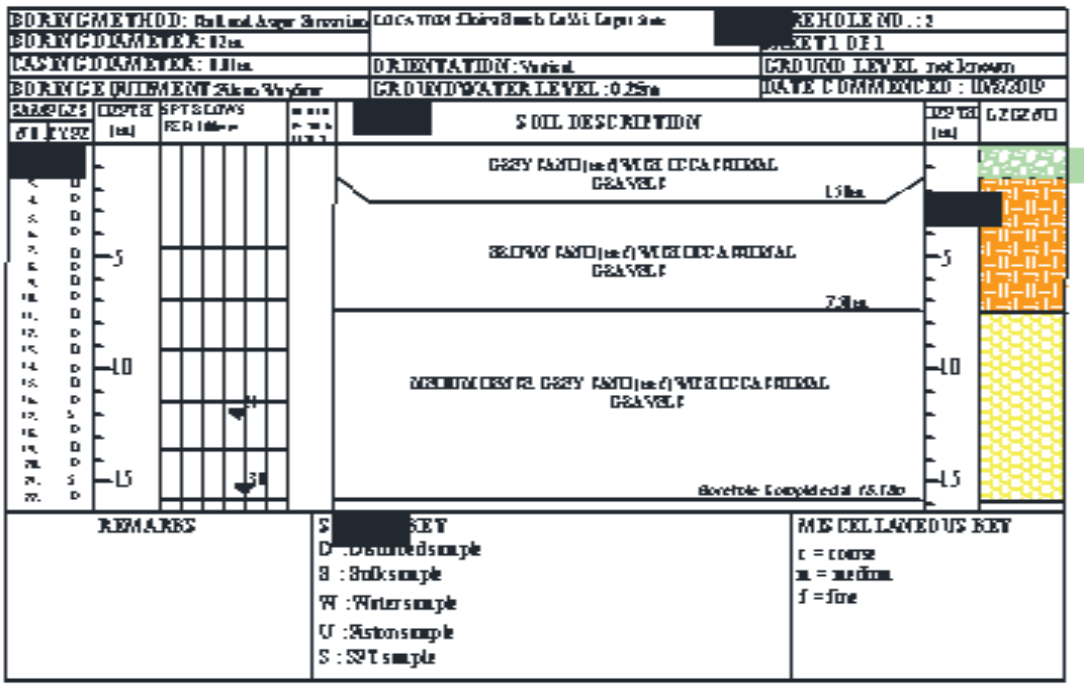

Figure 14b. Borehole log 2.

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3.4 Correlation of geophysical and geotechnical methods

The results of the geoelectric section AA^1, BB^1, CC^1, DD^1 and EE^1 signified topsoil with resistivity values ranging from 23.0 to 6356.9 Ωm within the depth range of 0.4 to 0.8 m, while the 2D result indicates topsoil with resistivity values ranging from 28 to 985 Ωm within the depth range of 0 to 5 m. Both results show that the topsoil is composed of clay, clayey sand/sandy clay, and sand brown/gray medium to fine sand with occasional gravels from the ground level to a depth of 1.5 m.

The second layer on all the geoelectric sections depict clay/peat, clay, sandy clay, sandy clay/sand, and sand having resistivity values ranging from 8.3 to 1157.2 Ωm and depth range of 1.8 to 5.4 m which corresponds to the 2D results signifying clay/peat, clay, clayey sand/sandy clay, and sand having resistivity values ranging from 8.5 to 985 Ωm to a depth of 10.0 m. While the second layer borehole log is an indicative of medium-dense, brown medium to fine sand with occasional gravels from depth of 1.50 - 7.50 m.

The third geoelectric layer denotes clay/peat, clay, clayey sand, sandy clay, and sand with resistivity values in the range of 9.8 to 284.1 Ωm within the depth range of 3.2 to 40.6 m which also corresponds with the 2D result indicating clay/peat, clay, clayey sand/sandy clay, and sand having resistivity values ranging from 8.5 to 148 Ωm to a depth of 20.0 m. Also, the third zone of the borehole logs revealed dense, gray medium to fine sand with occasional gravels from the depth of 7.50 – 15.75 m.

The fourth horizon on all the geoelectric sections represents the clay, sandy clay, clayey sand, and sand with resistivity values ranging from 10.3 to 473.3 Ωm within a depth of 8.4 to 57.1 m which also correspond with the 2D result indicating clay/peat, clay, clayey sand/sandy clay, and sand having resistivity values ranging from 8.5 to 329 Ωm to a depth of 40.0 m. The borehole logs could not go further due to the collapse of the sand

The fifth layer on all the geoelectric sections connote clay, sandy clay, sandy clay, and sand having resistivity values ranging from 16.8 to 401.0 Ωm and depth range of 25.9 to 34.4 m which corresponds to the 2D results signifying clay/peat, clay, clayey sand/sandy clay, and sand having resistivity values ranging from 3.0 to 329 Ωm to a depth of 50.0 m.

The sixth identify layer is symptomatic of sand having resistivity values ranging from 293.6 to 1007.8 Ωm. The depth range could not be determined due to the current termination within this zone. This shows that there are some degrees of correlation between the geophysical and geotechnical methods.

4. Conclusion

The electrical resistivity and geotechnical methods were deployed to characterize the subsurface geological parameters for pre-foundation building assessment at Oniru, Eti - Osa, Lagos State.

The integrated analysis of results from the VES and 2D imaging data reveal the soil to have similar layers namely topsoil, clayey sand, sandy clay, clay/peat, sandy clay/sand, and sand. The topsoil thickness values range from 0.4 to 0.8 m with its resistivity values ranging from 23.0 to 6356.9 Ωm, which corresponds with the first layer delineated on the borehole logs which is representative of clay, clayey sand/sandy clay, and sand brown/grey medium to fine sand with occasional gravels from the ground level to a depth of 1.5 m. The clayey sand layer has resistivity values ranging from 53.6 to 99.1 Ωm and layer thickness of 7.6 to 25.9 m. The resistivity values of the sandy clay layer range from 51.1 to 93.1 Ωm, having thickness values from 1.9 to 14.8 m. The sandy clay/sand has resistivity values ranging from 104.7 to 1157.2 Ωm and layer thickness of 1.8 to 5.2 m. The borehole logs correspond to this layer having an N-Value of 22 to 30 which is indicative of medium-dense, brown medium to fine sand with occasional gravels from the depth of 1.50 - 7.50 m. The clay layer has resistivity values ranging from 10.3 to 46.3 Ωm and thickness ranging from 2.2 to 57 m. The clay/peat has resistivity values ranging from 8.3 to 9.8 Ωm and layer thickness of 2.9 to 11.0 m. The sand in VES (3 to 15, 17, and 19) possesses resistivity values ranging from 108.8 to 1007.8 Ωm and layer thickness of 4.3 to 24.4 m. The thickness in VES (1 to 3, 6, 8, 10, 16 and 19 to 25) could not be determined due to current termination. The information obtained from the two borehole logs in the area correlates significantly with the geophysical results for the layers which connotes dense, grey medium to fine sand with occasional gravels at the

depth range of 7.50 – 15.75. The analysis of the geophysical and geotechnical study shows that the soil in the study area is made up of sand and clay, but the sand is more pronounced at depth intervals of 1.5 to 20 m.

In addition, the sand zones from the study represent a competent layer that can withstand engineering structures. This is because of the thickness of the sand layers and the ability of sand as an engineering material to absorb water without expansion or contraction during the dry period, unlike clay and peat that has high ability to expand and contrast during wet and dry periods.

Lastly, the differences in the electrical properties of the different soil samples available in this location occurred because of the difference in material and mineral compositions of the particles that made up these soil materials. Moreover, some of those soil layers are the same kind but they are found at different depths in this location, this is because of the differences in moisture and salinity content. The area under investigation is located along the coastline part of Nigeria with high levels of saline water intrusion from the coast into the surroundings.

Acknowledgement

`The authors are grateful to Mr. Taofik Adeleke and his team at Lukado Consults Limited and The Earth Signature Research Group (ESReG) for their assistance during this work.

Conflict of Interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Publication Ethic

Submitted manuscripts has not been previously published by or be under review by another print or online journal or source.

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Road Construction and Vehicular Activities as Indicators for Heavy Metal Pollution in Osogbo Metropolis, South West Nigeria

Wasiu Bolade Agbaje[1*], Mutiu Alani Fakunle[2], Luqman Adeyemi Azeez[1], Olalekan Aderemi Olabode[1], Oluwadurotimi Olutosin Akintade[3]

[1]Department of Pure and Applied Chemistry, Faculty of Basic and Applied Sciences, Osun State University, Osogbo, Nigeria
[2]Department of Physics, Faculty of Basic and Applied Sciences, Osun State University, Osogbo, Nigeria
[3]Department of Chemical Sciences, Faculty of Applied Sciences, KolaDaisi University, Ibadan, Nigeria
***Corresponding author e-mail: wasiu.agbaje@uniosun.edu.ng, agbajewb@gmail.com**

Received: *10 August 2022* / **Revised:** *20 October 2022* / **Accepted:** *20 December 2022*

Abstract

The levels of heavy metals in soil samples from selected major roads under construction, burrow sites (a place where soil was obtained for road filling) and two roads under use have been investigated. Soil samples were collected and digested using standard methods. The digests were analyzed for Ni, Cr, Zn, Cd, Cu and Pb using Atomic Absorption Spectrophotometer. The data were subjected to descriptive statistics, non linear regression and one way analysis of variance (ANOVA). The results showed that cadmium had the highest Contamination Factor (CF). The Pollution Load Index of the roads under construction was higher than each of the Burrow site and one of the Trunk C roads analyzed for comparison. The Ecological Risk Factor of the sampling sites was less than 40 which indicated that, the levels of heavy metals on the road construction sites did not pose ecological risks to the environment. The Ecological Risk Index of each of all the sampling sites was less than 150 indicating that they fell within the low ecological risk category. The study established that the Burrow site did not contribute to the heavy metal concentration of the road under construction and the contamination was traced to vehicular activities.

Keywords: Heavy metal concentration, City pollution, Ecological risk factor, Burrow site, Vehicular density

1. Introduction

Heavy metals are generally regarded as substances with density five times higher than that of water, and can have adverse effects on animals and plants (Jarup, 2003). These metals are indestructible and many are toxic if present beyond permissible levels and thus, heavy metal pollution of the natural environment is a global issue. According to Kirpichtchikova et al. (2006), most metals are not degraded by microbes or chemical means but are oxidized in the presence of moisture and oxygen to cations which persist for a long period in soils. It has been reported that heavy metal pollution affects crop quality and production, water bodies, and threatens human and animal health via accumulation through the food chain, inhalation of air and skin contact (Satarug, Garrett, Sens, & Sens, 2010). These metals can be taken up by plants and animals from soil, air and water sources. In plants, they are absorbed along with water through the stomata or by the root hairs (Satarug et al., 2010). They may have stimulatory, toxic or inhibitory effects on biochemical processes in plants and animals (Gikas & Romanos, 2006).

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Such effects in humans include altering DNA and RNA, reproductive system disorders, cancer, bone mineralization, Parkinson's disease etc. High concentrations of heavy metals in soil have been reported to have caused a number of deleterious effects on plants which include growth retardation, destruction of chlorophyll, disorders in biochemical activities, mutations and reproductive disorders (Gall & Rajakaruna, 2013).

Road construction has remained the major activity for the growth of industrial units, stimulating economic activities and this has led to the loss of forest cover and subsequent loss of soil fertility. Roadside soils often show an elevated amount of contamination that can be credited to automobiles (Mmolawa, Likuku, & Gaboutloeloe, 2011). The discharge of heavy metals is one of the most important environmental issues caused by road construction (Mafuyai, Kamoh, Kangpe, Ayuba, & Eneji, 2015). Erosion by wind and water facilitates the entry and spread of these metals in the environment (Morais, Costa, & Pereira, 2012). Indeed, heavy metals have been reported to receive much attention due to their toxicity (Su et al., 2012) and their presence is a useful pointer for contamination in soils (Ubwa, Abah, Ada, & Alechenu, 2013).

Heavy metal status of soils from some major roads in Nigeria has been reported, they include Ogbomoso, South West Nigeria (Yekeen & Onifade, 2012), Maiduguri (Uwah & John, 2014), Jos Metropolitan Area, Nigeria (Mafuyai et al., 2015) and in Parts of Owerri, Nigeria (Okereke, Nduka, Ukaoma, & Ogidi, 2019). Similar reports have been obtained in roadside soils along the Shenyang-Dalian highway in Liaoning Province, China (Hui et al., 2017). Similar studies have not been obtained in Osogbo in recent times. Indeed, due to large population and rapid urbanization, most people in Osogbo live close to busy roads where they can be exposed to metal pollution from various activities on the roads. It is therefore imperative to investigate the heavy metal status in top-soils of selected roads under construction in Osogbo metropolis, as an indicator for pollution of selected areas in Osogbo, the capital of Osun State, Nigeria.

2. Materials and Methods

2.1 Sampling sites and sample collection

The city of Osogbo, the capital of Osun State, is situated between latitude 7º 6' N and 7º 15' N, and longitude 3º 17' E and 3º 25' E; and covers about 268 km^2. It is located in the south-western part of Nigeria and about 100 km south of Ilorin, 115 km northwest of Akure and 88 km northeast of Ibadan. The capital city comprises of Olorunda and Osogbo Local Government Areas with a total population of 300,000 people (Oyelowo, Chima, & Oladoye, 2010; Taiwo, Michael, Gbadebo, & Oladoyinbo, 2019; Tijani & Onodera, 2009). Osogbo, being the capital of the State, is the focus of a large number of migrants due to political and socio-economic activities.

The criteria for the selection of roads for the study area were based on the major road constructions being undertaken. The study Road Stretches (RS) represented alphabetically as A to F were: Ilesa Garage–Suzzy (RSA**)**, Suzzy–Okebale Roundabout (RSB**),** Okebale Roundabout–Testing Ground (RSC**)**, Testing Ground–Stadium Roundabout (RSD**)**, Olorunkemi– Suzzy (RSE) and UNIOSUN –Sasa (RSF) as well as the burrow site (G). A, B, C and D were Roads under construction with simultaneous vehicular movement (D > A > C > B); E was a trunk C road with very low vehicular activities; F was also a trunk C road but with high vehicular activities involving commercial and private vehicles; G was a burrow site, i.e., the place where the construction companies normally obtain soil to fill the roads. Road E and F were used as controls.

A total of eighty eight (88) samples were taken from the aforementioned areas of study with seventeen from RSA, four from RSB, ten from RSC, five from RSD, nine from RSE, twenty three from RSF and twenty from G. The soil samples

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were obtained with plastic spoons at a depth of about 0.5 cm from the surface of the roads. The sampling points and locations are as presented in Figure 1. The samples were air–dried at a clean section of the laboratory, sieved through 2 mm mesh and placed in white plastic bags. Samples from each road stretch were combined and labelled appropriately; RSA, RSB, RSC, RSD, RSE and RSF were labelled as samples A, B, C, D, E and F, respectively while the combined samples from burrow site was labelled as sample G. They were all kept at a dry place pending the analysis.

2.2 Sample digestion

Wet digestion method was used as earlier described by Addis and Abebaw (2017). All reagents used were of analytical grade and distilled–deionized water was used for rinsing and sample preparation. Soil sample (air–dried, ground, and sieved) of 1.0 g was weighed into a digestion tube. Aqua regia of 12 mL and 3 mL of H_2O_2 were measured and added into the digestive tube and gently swirled to mix the sample properly. The digestion tubes were then placed on a digestion furnace (Surgifield Medical, Model SM1008,

England, UK) and heated at 180°C for 3 h inside a fume cupboard. All the digests were cooled, filtered through Whatman No 42 filter paper and diluted to 100 mL with distilled–deionised water. Each sample was digested in replicates of three, transferred to acid–washed stoppered glass bottle, labelled and kept for analysis with Atomic Absorption Spectrophotometer (AAS – PG990, United Kingdom). The concentrations of, Cd, Pb, Ni, Cr, Zn and Cu in the samples were then determined. The blank was prepared in a similar way using distilled–deionized water. The instrument working calibration was made by diluting commercial Scharlau Japan stock solution (1000 ppm) standard with distilled–deionized water. A recovery study was carried out by spiking portions of the samples with known amounts of the analytical standards of cadmium, lead, nickel, chromium, zinc and copper. All of these were dried, homogenized, and passed through the digestion and analytical steps. The mean percent recoveries were determined by dividing the experimental concentration with the spiked concentration for each of the metals.

2.3 Data analysis

The measurements were done in triplicate. Data were subjected to descriptive statistics, non linear regression and one way analysis of variance (ANOVA). Duncan's multiple range test (IBM SPSS Statistics 20) was carried out to establish the significant level at $P < 0.05$. Results were expressed as means ± Standard Deviation (SD). In addition, the Contamination Factor (CF) for the metals were calculated and used to assess the extent of contamination at each of the sampling sites. The CF for a particular metal is the ratio of the concentration of the metal in the soil to the concentration of the background or target value. CF is a good index for contamination assessment and to guide decision making (Yahaya, Abubakar, & Abdu, 2021).

The pollution load index (PLI) at each of the sampling site was also calculated to assess the combined metal concentration at the sampling site and it took into consideration the contribution (sum) of the contamination factors of each metal at a study site. It is suitable for site assessment and decision making (Yahaya et al., 2021). Furthermore, the Ecological Risk Factor (ERF) was determined to evaluate the potential ecological risk associated with the presence of each metal. It was obtained as a product of the individual toxic response factor and contamination factor (Yahaya et al., 2021). The Potential Ecological Risk Index (PERI) was calculated to determine the ecological risk at each sampling site. It was obtained as the cumulative effect of the ecological risk factor of each metal. It is a good index for site quality assessment (Yahaya et al., 2021).

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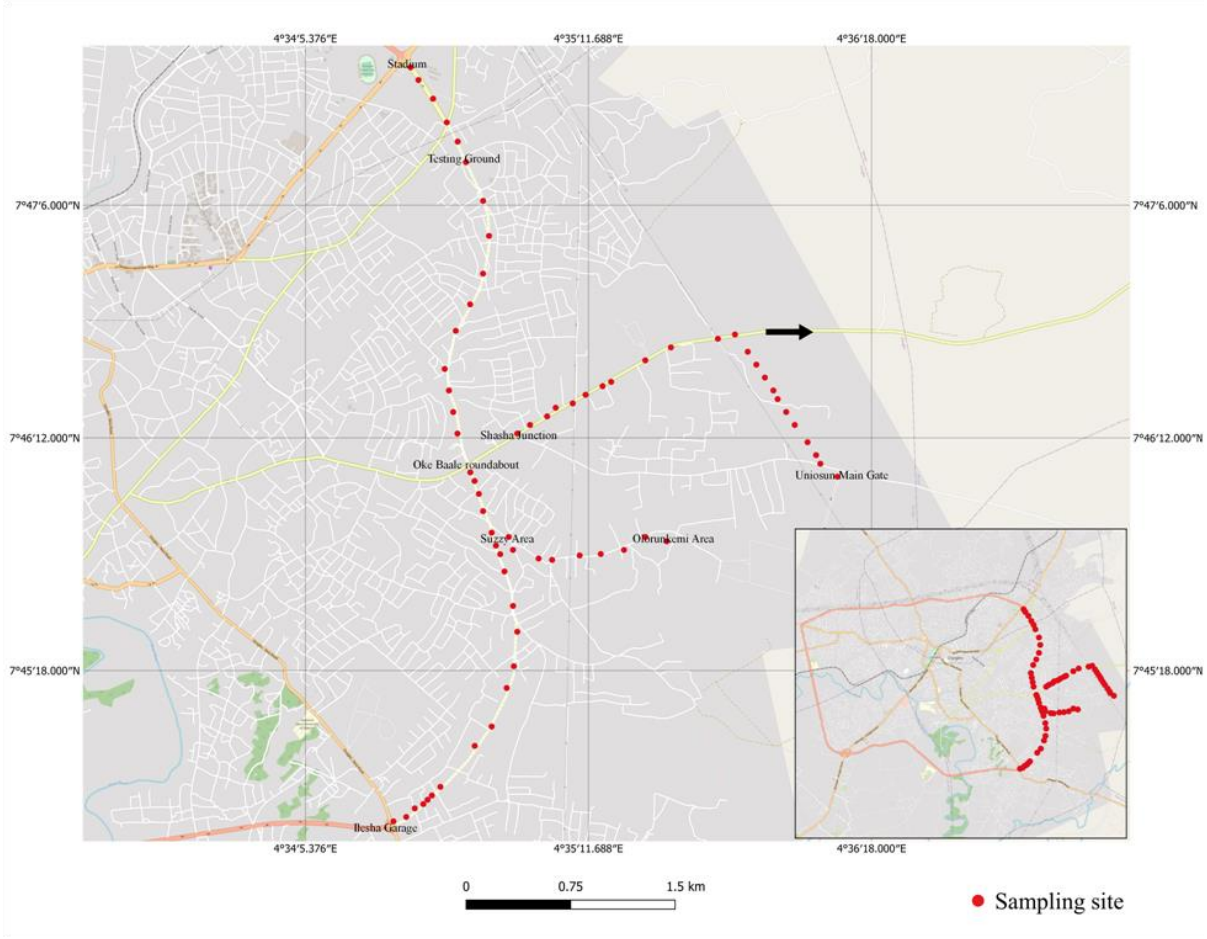

Figure 1. Map of the roads under construction showing the sampling location.

3. Results and Discussion

3.1 The levels of heavy metals

The concentrations of the heavy metals, their ranges and comparison of their average values with the levels reported for other countries are as shown in Table 1, Table 2 and Table 3, respectively. The concentration of Nickel in the samples ranged from 7.22 to 15.15 mg/kg and the least and highest values were obtained in samples E (Olorunkemi–Suzzy road) and C (Okebaale Roundabout – Testing Ground), respectively. The levels were significantly different ($P < 0.05$). Low concentration of Nickel is expected from sample E, although it is under usage, but only a few numbers of vehicles ply it. Sample C with the highest level of Ni is not significantly different from sample D (Testing Ground – Stadium Roundabout). Both roads were under construction and the reason for the high level could be attributed to private and commercial vehicular activities that took place

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simultaneously with the construction work. The level of Nickel in each of samples C and D was significantly higher than the concentration recorded in sample G. As shown in Table 3, comparison of the average levels of Nickel in all the samples showed that it was lower than the levels reported for other countries as well as the permissible levels by United States Environmental Protection Agency (USEPA) and Department of Petroleum Resources (DPR) in Nigeria. Low level of Nickel is of advantage because it has been reported that Nickel is a carcinogenic agent that can also induce systemic reactions (Suzuki, Yabuki, & Ono, 2008). Report indicated that Nickel finds its way into the ambient air as a result of the combustion of coal, diesel oil and fuel oil (Cempel & Nikel, 2006).

The chromium contents of the soil samples ranged from 14.41 mg/kg (Sample G) to 46.08 mg/kg (Sample D). The values were significantly different ($P < 0.05$). Thus, the levels of Cr in samples B, C, E and G were not significantly different ($P < 0.05$) and were all significantly lower than those in samples A, D and F. The chromium contents of the roads under construction (Samples A, D and F) were significantly higher than that of burrow site (sample G); this could be attributed to private and commercial vehicular activities that took place simultaneously with the construction work. Chromium content of sample E is expected to be significantly lower than sample F ($P < 0.05$) based on fewer and large numbers of vehicles that ply them, respectively. Comparison of the average chromium contents with other countries (Table 3) showed that, the level recorded in this study was less than the levels in Asia and Europe. Comparison with international standards showed that the level of chromium in this study was less than National Environmental Standards and Regulations Enforcement Agency (NESREA), WHO and DPR, but the value was almost twice the tolerable level by USEPA. Higher level of chromium is dangerous to humans because it has been linked to cancer pathogenesis (Liu, Shen, Liu, Wang, & Li, 2007).

The zinc contents of the samples ranged from 4.25 mg/kg in sample E to 30.24 mg/kg in sample F. The levels of zinc in the samples were significantly different ($P < 0.05$). Highest level of zinc in sample F could be attributed to vehicular density. The levels of zinc at the burrow site (sample G) and sample D were not significantly different but were significantly lower than level of zinc in sample F. Thus, the levels of zinc in the samples varied from one sample to the other and the variation was in line with vehicular activities on the roads outside and around the construction site. The average level of zinc in this study was lower than values from other countries as well as local and international regulatory bodies (Table 3). Zinc is a raw material in the production of brake lining because of its heat conducting properties. Consequently, its particles could be released due to continual mechanical abrasion of the vehicles. Also zinc particles could be released to the environment due to combustion processes of engine oil and motor vehicle tire attrition (Shinggu, Ogugbuaja, Barminas, & Toma, 2007). It has been asserted that among the heavy metals, Zn is the least toxic and very essential component of healthy diet; however beyond the tolerable limit, for an instance zinc sulfate tablets, containing 150 mg elemental zinc, it could lead to health-related complications such as diarrhea, fever, nausea, lethargy and vomiting (Fischer, Giroux, & L'Abbe, 1984; Samman & Roberts, 1987).

The concentrations of cadmium in the samples ranged from 0.24 mg/kg in sample G to 1.40 mg/kg in sample D, and were significantly different ($P < 0.05$). The Cd contents of samples E and G were not significantly different ($P < 0.05$) and the value was the least among the samples. Sample D had the highest concentration of Cd. The average level of Cd in this study was higher than the tolerable levels in Asia but lower than the tolerable levels in Europe and UK. Comparison with local and international regulatory bodies showed that the value was higher than the tolerable level approved by WHO and USEPA. The level was however

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lower than 3 mg/kg from NESREA/FEPA, but comparable with 0.8 mg/100g from DPR. Cadmium in the samples could be caused by combustion of lubricating oil and wearing of tires from road abrasion (Deska, Bombik, Marciniuk-Kluska, & Rymuza, 2011). According to Ahmed et al. (2015), cadmium is a classic carcinogen which could lead to hepatic, renal, and pulmonary injury.

The copper contents of the samples ranged from 6.72 mg/kg in sample E to 20.43 mg/kg in sample D. The values were significantly different at $P < 0.05$. The copper content of samples G and E (Olorunkemi–Suzzy) were not significantly different but were significantly lower than the copper contents of other samples ($P < 0.05$). Obviously, low vehicular movement must have contributed to the low level of Cu in sample E and lack of vehicular movement must have plausibly accounted for the low level of copper at the burrow site. Just like the majority of other heavy metals that were considered in this study, the highest level of copper was obtained in sample D. Thus, apart from Ni and Zn, where samples C and F had the highest concentrations, respectively, sample D had the highest levels of Cr, Cd and Cu. Comparison of the average levels of copper in all the samples showed that they were lower than reported values from other countries, and both the national and international regulatory bodies. Presence of Cu in soil samples has been reported by Akbar, Hale, Headley and Athar (2006) to be a reflection of activities involving either engine wear, thrust bearings, bushing or bearing metals.

The recovery of each of the metals was higher than 90%.

3.2 Contamination factor

It has been proved that the contamination factor of a metal has a direct proportionality to the extent of pollution and or contamination of that metal (Lacatusu, 1998). According to the author, when the contamination factor is lower than 1, it indicates lower risk, while values greater than 1 denote higher risks. Accordingly, the contamination factors for the heavy metals in each of the samples at all the sampling sites are as shown in Figure 2. The contamination factor of each of the various sampling sites was less than 1 except sites C and F. This implies that road C (under construction with simultaneous vehicular movement) and F (a trunk C road, busy because staff and commercial vehicles ply it) were slightly polluted with Cd whereas the other sampling sites fell within the range of very slight contamination to very severe contamination for all the heavy metals. None of the roads was severely or excessively polluted as a result of road construction activities. However, the contamination factor of Cd was found to be higher than those of the other heavy metals at all the sampling sites.

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Table 1. Concentration of heavy metals in the soil samples (mg/kg).

Samples	Ni	Cr	Zn	Cd	Cu
A	10.43 ± 2.81^{b}	23.31 ± 14.77^{b}	15.42 ± 7.93^{b}	1.08 ± 0.19^{c}	10.69 ± 6.59^{b}
B	11.76 ± 2.16^{b}	14.60 ± 1.43^{a}	20.75 ± 3.17^{c}	0.58 ± 0.23^{b}	9.32 ± 4.01^{ab}
C	15.15 ± 0.70^{c}	14.88 ± 0.14^{a}	15.67 ± 0.23^{b}	0.69 ± 0.01^{b}	7.20 ± 0.09^{a}
D	14.68 ± 0.45^{c}	46.08 ± 0.11^{c}	18.98 ± 5.86^{ab}	1.40 ± 0.09^{c}	20.43 ± 0.82^{c}
E	7.22 ± 7.25^{a}	14.72 ± 14.24^{a}	4.25 ± 3.77^{a}	0.24 ± 0.24^{a}	6.72 ± 5.77^{a}
F	10.35 ± 0.16^{b}	20.54 ± 0.61^{b}	30.24 ± 0.16^{d}	0.54 ± 0.06^{b}	10.35 ± 0.12^{a}
G	7.84 ± 3.76^{a}	14.41 ± 15.61^{a}	18.09 ± 18.81^{ab}	0.24 ± 0.42^{a}	7.32 ± 8.91^{a}
Total Mean	11.06 ± 4.06	21.22 ± 13.64	17.63 ± 10.07	0.68 ± 0.46	10.29 ± 6.13
% Recovery	91.34 ± 10.5	100.00 ± 2.7	95.50 ± 4.7	94.75 ± 7.01	98.66 ± 3.34

The results are presented as means ± SD for three samples (n = 3).

$^{a-d}$ Means followed by different letters on the same column are significantly different at $P < 0.05$.

Samples - A: Ilesa Garage–Suzzy, **B:** Suzzy–Okebale Roundabout**, C:** Okebale Roundabout–Testing Ground, **D:** Testing Ground–Stadium Roundabout, **E:** Olorunkemi–Suzzy, **F:** UNIOSUN–Sasa, G: Burrow Site

A, B, C, D: Roads under construction with simultaneous vehicular movement

E: Control (a trunk C road, not busy because few vehicles ply the road)

F: Control (a trunk C road, busy because staff and commercial vehicles ply it)

G: (burrow site, i.e., the place from where the construction companies obtain soil to fill the road)

Table 2. The range of the concentrations of heavy metals in each of the soil samples (mg/kg).

Samples	Ni	Cr	Zn	Cd	Cu
A	9.32–13.62	11.50–39.87	7.60–23.45	0.90–1.28	6.74–18.30
B	9.44–13.72	13.12–15.98	17.42–23.72	0.34–0.80	6.91–13.95
C	14.52–15.9	14.73–15.01	15.40–15.81	0.68–0.70	7.10–7.25
D	14.18–15.05	46.00–46.21	15.30–25.74	1.30–1.46	19.91–21.37
E	7.15–14.5	5.52–7.22	5.52–7.22	ND–0.72	0.58–12.02
F	10.21–10.53	20.00–21.20	30.10–30.42	0.49–0.60	10.21–10.43
G	4.22–11.72	2.1–31.97	4.08–39.47	ND–0.73	1.47–17.57

Samples – A: Ilesa Garage–Suzzy, **B:** Suzzy–Okebale Roundabout**, C:** Okebale Roundabout–Testing Ground, **D:** Testing Ground–Stadium Roundabout, **E:** Olorunkemi– Suzzy,

F: UNIOSUN–Sasa, **G:** Burrow site

A, B, C, D: Roads under construction with simultaneous vehicular movement

E: Control (a trunk C road, not busy because a few vehicles ply it)

F: Control (a trunk C road, busy because staff and commercial vehicles ply it)

G: Burrow site, i.e., the place where the construction companies obtain soil to fill the road

ND: Not Detected

3.3 Pollution load index

The pollution load index gives an indication of the extent of contamination at the sampling sites by taking into account the individual contributions of the metals. The pollution load index for each of the study site is less than 1 as shown in Figure 3 and followed a descending order: F > C > B > D > E > G > A. Each of the roads under construction (B, C and D) had a higher pollution load than the Burrow site (G) and road E used for comparison. However, road F which was not under construction at the time of this study had a higher pollution load than roads A, B, C, D and E. This might have been due to the high traffic density on road F from the commercial and staff vehicles.

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Table 3. Comparison of the total average levels (mg/kg) of the heavy metals in this study with levels from other countries and tolerable levels from national/international regulatory bodies.

Country/Regulatory bodies	Ni	Cr	Zn	Cd	Cu	Reference
Osogbo, Nigeria (all sites mean)	11.06	21.22	17.63	0.68	10.29	This study
Asia (CSEPA)	40	150	200	0.3	50	Hu et al., 2017
Europe (Germany)	200	500	600	5	200	He et al., 2015
UK	230	N/A	N/A	1.8	N/A	He et al., 2015
NESREA/FEPA	70	100	421	3	100	Ogbonna et al., 2020
WHO		100		0.1	100	Ogbonna et al., 2020
USEPA	72	11	1100	0.48	270	He et al., 2015
DPR	35	100	140	0.8	36	DPR, 2002

NESREA/FEPA: National Environmental Standards and Regulatory Enforcement Agency/Federal Environmental Protection Agency

DPR: Department of Petroleum Resources

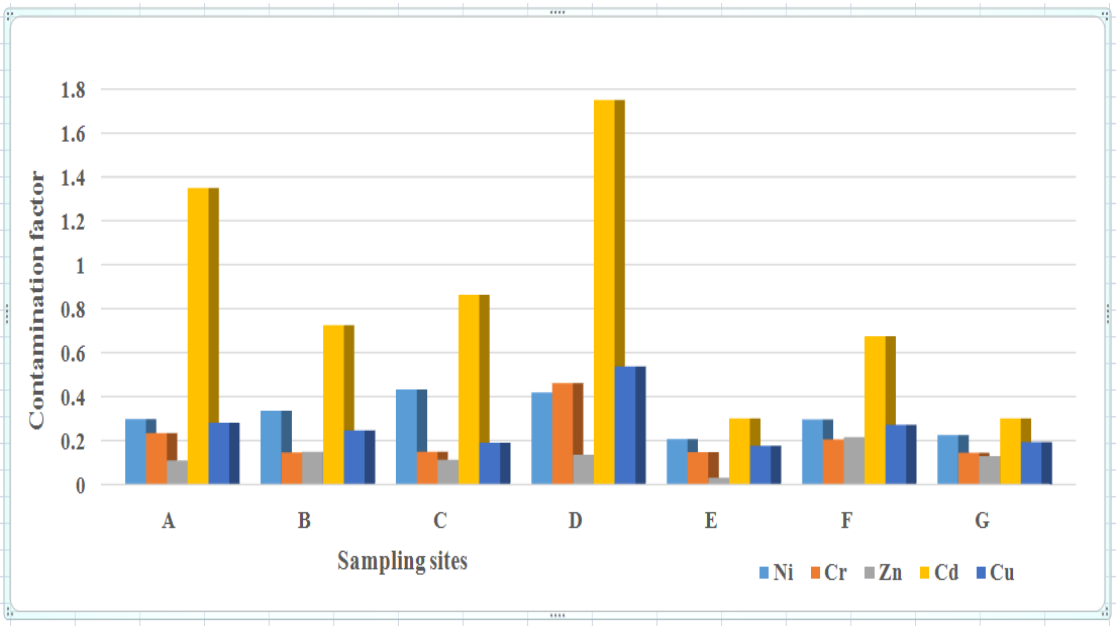

Figure 2. Contamination factor for the heavy metals at the sampling sites.
Samples – **A**: Ilesa Garage–Suzzy, **B**: Suzzy–Okebale Roundabout, **C**: Okebale Roundabout–Testing Ground, **D:** Testing Ground–Stadium Roundabout, **E**: Olorunkemi–Suzzy, **F:** UNIOSUN–Sasa, **G**: Burrow Site

3.4 Ecological risk assessment

3.4.1 Ecological risk factor

The results obtained for the ecological risk factor are shown in Figure 4. The ecological risk factor for all the heavy metals was found to be lower than 40. This implies that the presence of the metals on the roads posed low ecological risks. Cd had the highest ecological risk factor at all the sampling sites. This result was consistent with the results obtained for the contamination factor of the metals at the various sites.

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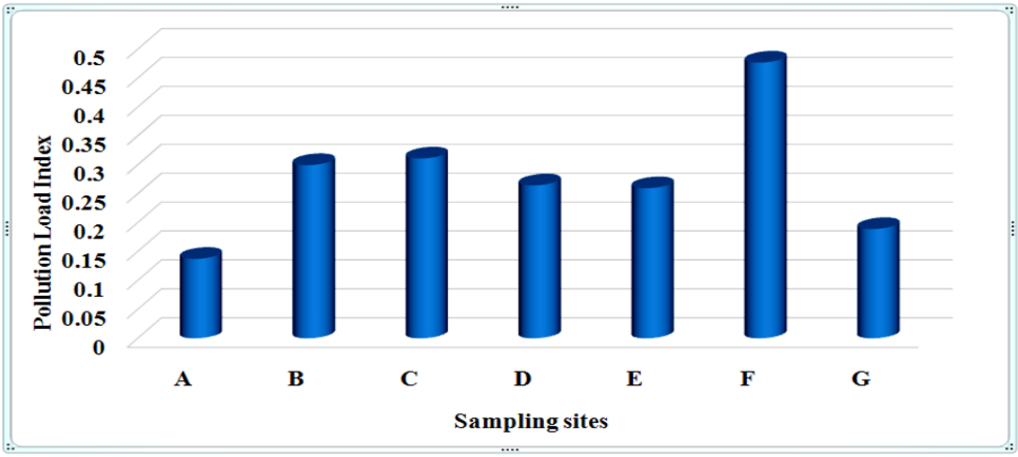

Figure 3. Pollution Load Index for the metals at the sampling sites.
Samples – **A**: Ilesa Garage–Suzzy, **B**: Suzzy–Okebale Roundabout, **C**: Okebale Roundabout–Testing Ground, **D**: Testing Ground–Stadium Roundabout, **E**: Olorunkemi–Suzzy,
F: UNIOSUN–Sasa, **G**: Burrow site

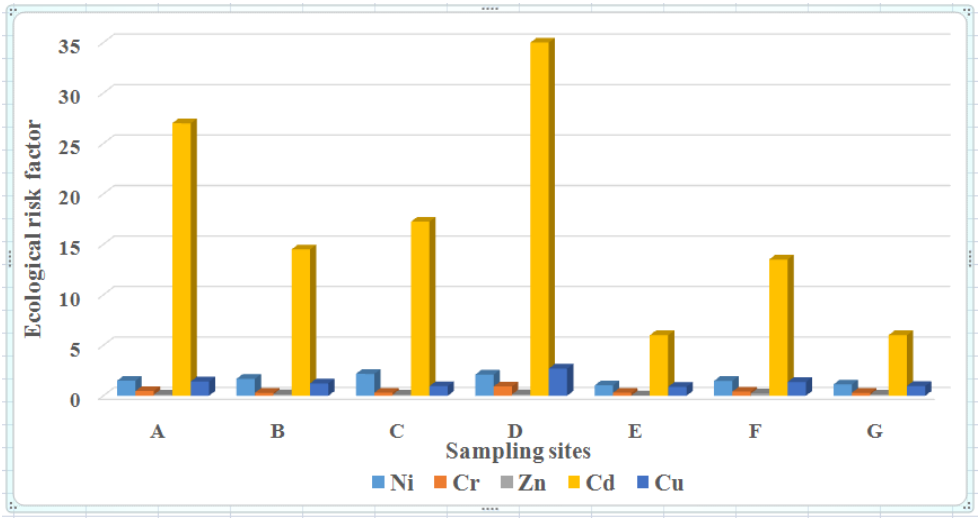

Figure 4. Ecological risk factor for the metals at the various sampling sites.
Samples – **A**: Ilesa Garage–Suzzy, **B**: Suzzy–Okebale Roundabout, **C**: Okebale Roundabout–Testing Ground, **D**:Testing Ground–Stadium Roundabout, **E**: Olorunkemi–Suzzy,
F: UNIOSUN–Sasa, **G**: Burrow Site

3.4.2 Potential ecological risk index

The result obtained for the potential ecological risk index is as shown in Figure 5. The potential ecological risk index takes into account the contributions of each of the heavy metals and provides a broader picture of the extent of contamination at each of the sampling sites. From the result obtained, the potential ecological risk index of the roads followed a descending order: F > C > E >D. Thus, road F (used for comparison) had the highest value which was even higher than the roads under construction (A, B, C and D) and the Burrow site (G) as well as road E that was also used for comparison. However, each of the values

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lied within the low ecological risk category (< although, analysis of the results from this study showed that none of the sampling sites would pose risk to the people, however, the level of cadmium with an average level that was higher than the tolerable level approved by WHO and USEPA called for worry and concern. This is attributed to the fact that heavy metals have potential to accumulate and worst still, they have been reported to remain in soil for extended periods because they degrade slowly, posing a severe risk to ecosystems and threatening human health through exposure pathways such as inhalation of dust, dermal contact, and ingestion of crops grown in the soil. Indeed, vehicular emission is needed to be under serious control. Results from a study by Kristensson et al. (2004) revealed that gaseous emissions were higher in Sweden than in the USA and Switzerland, foremost due to the lower-fraction catalytic converters in Sweden.

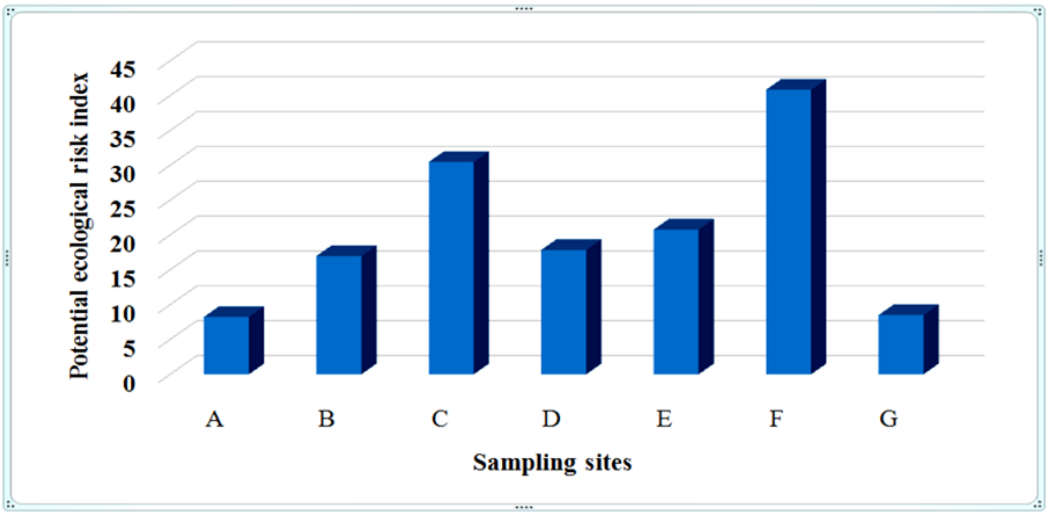

Figure 5. Potential ecological risk index of the metals at the sampling sites.
Samples – **A:** Ilesa Garage–Suzzy, **B**: Suzzy–Okebale Roundabout, **C**: Okebale Roundabout–Testing Ground, **D:** Testing Ground–Stadium Roundabout, **E**: Olorunkemi–Suzzy, **F:** UNIOSUN–Sasa, **G:** Burrow site

4. Conclusion

The study established the levels of heavy metals in the samples and analysed the influence of the generated data on the inhabitants of the area where the construction work took place with respect to contamination, pollution and ecosystem. The results showed that cadmium had the highest concentration and its average level was higher than the tolerable level approved by WHO and USEPA. The UNIOSUN access road had the highest contamination factor with respect to all the heavy metals investigated although it was not under construction at the time of this study. The UNIOSUN access road also had a higher pollution load than each of the roads under construction, the Burrow site and Olorunkemi–Suzzy (a control) which might have been due to the high vehicular density from the commercial and staff vehicles. The ecological risk factor for all the roads were found to be lower than 40 implying that, the presence of the heavy metals on the roads posed low ecological risks to the society. The Potential Ecological Risk Index (PERI) of each of the sites lied within the low ecological risk category (< 150) indicating that none of the sampling sites would pose risk to the people. It is however advisable to check the status of the soil at the burrow sites before they are approved to fill the road. Also vehicular emission should be controlled to check the pollution load, contamination factor and ecological risk index.

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Bacteriocin from *Bacillus velezensis* BUU004 as a Seafood Preservative: Antibacterial Potential, and Physical and Chemical Qualities of Dried, Seasoned, and Crushed Squids

Pornpimon Soodsawaeng[1], Nanticha Rattanamangkalanon[2], Traimat Boonthai[1], Verapong Vuthiphandchai[3], Subuntith Nimrat[2]*

[1]Biological Science Program, Faculty of Science, Burapha University, Chon Buri 20131, Thailand
[2]Department of Microbiology, Faculty of Science, Burapha University, Chon Buri 20131, Thailand
[3]Department of Aquatic Science, Faculty of Science, Burapha University, Chon Buri 20131, Thailand
***Corresponding author e-mail: subunti@buu.ac.th; subunttbt@gmail.com**

Received: *22 September 2022* / **Revised**: *15 November 2022* / **Accepted:** *19 December 2022*

Abstract

Food safety of seafood-based products has become an important health threat in Thailand. Simultaneously, potential hazards posed by the use of chemical preservatives have prompted the advent of alternative technologies. Bacteria-derived substances have attracted interest as biopreservative to respond to health conscious demand of consumers. In order to enhance biosafety quality from a farm to fork cycle of traditional seafood products, bacteriological assessment during multiple steps related to preparation of dried, seasoned and crushed squids was conducted. Total viable counts (TVC) in the ranges of 10^2 - 10^3 CFU/g were observed across a series of the preparation from rinsing to 2nd sun-dry phases. Homemade seasoning sauce composed mainly of Thai spices and flavoring was the common source of spoilage bacteria supported by the highest TVC population and diversity. Three bacterial genera belonging to *Bacillus*, *Kocuria* and *Staphylococcus* existed predominantly in the prepared squids. In the subsequent phase of study, antibacterial potential, and mode of action of a semi-purified solution containing bacteriocin from *B. velezensis* BUU004 (SPS-BV) against pathogenic *B. cereus* were investigated. The SPS-BV (800 AU/mL) exhibited strong bactericidal activity towards *B. cereus* through cell lysis. Lastly, biopreservative potential of the SPS-BV was evaluated in the post-prepared dried squids during 28-day storage at room temperature. The SPS-BV was as effective as commercial nisin for controlling food spoilage bacteria along with significant reductions in moisture content and a_w of dried squids during storage. This study confirms the biopreservative potential of the SPS-BV in dried seafood products in Thailand.

Keywords: Biopreservative, *Bacillus*, Seafood, Food safety, Dried squid

1. Introduction

Seafood and seafood products has been a relevant part of the community in Thailand because of being important source of proteins, essential amino acids, omega-3 fatty acids, vitamins, and minerals, and providing health benefits in prevention of life-threatening events, e.g. cardiovascular diseases and rheumatic diseases (Venugopal, 2018). Traditional dried seafood-based products are one of the most consumed categories of ready-to-eat (RTE) foods in Thailand due to a busy lifestyle of working individuals, the convenience offered, time-saving, and the little effort required for the preparation. A large part of preparation generally uses wild-caught fish and shellfish as raw materials resulting in the control of certain hazards very difficult in some traditional RTE seafood. As a consequence, consumption of traditional RTE seafood is not risk-free. Seafood represents a high risk of public health with common implication in foodborne outbreak in Thailand accounting for 27.5 and 33.3% of strong-evidence events in 2012 and 2019 (Donla, Junthepa, & Promsiri, 2019; Poonawagul & Jearanaiwongkul, 2012). Traditional RTE seafood products available in a local market in Chon Buri province, Thailand have been reported to contaminate with spoilage bacteria over the acceptable limit of total viable bacteria in processed products (< 5 x 10^5 CFU/g) and cooked products (< 5 x 10^4 CFU/g) imposed by Department of Fisheries

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(2009). Foodborne pathogens, e.g. *Bacillus cereus*, *Escherichia coli*, and *Salmonella* are also existed in the RTE seafood products (Butkhot et al., 2019a; Nimrat et al., 2019; Thungkao & Muangharm, 2008). An improvement of biosafety quality of the RTE seafood products is required through inhibiting the growth of spoilage and pathogenic bacteria. Chemical preservatives have been extensively used in traditional RTE seafood products to eliminate undesirable bacteria. However, a health concern caused by the overuse of chemical additives currently become an important raised issue because some of them are expected to involve in deteriorative health effects, e.g. intoxications, allergies, cancers, hepatotoxicity, and teratogenicity (Zhao et al., 2019). Therefore, there is a great increasing interest in the use of saprophytic bacteria and/or their metabolites as a novel, safe, and food-compatible biopreservative for inhibiting pathogens and extending shelf-life of seafood products.

In the context of food safety, members of *Bacillus* genus, a Gram-positive, aerobic, and endospore-forming bacterium with rod-shaped morphology, have a long history of safe use simultaneously with a potential probiotic application in human and animal foods. Some of the main representatives within *B. subtilis* complex, e.g. *B. amyloliquefaciens*, *B. atrophaeus*, *B. velezensis*, *B. licheniformis*, *B. methylotrophicus*, *B. mojavensis*, *B. siamensis*, *B. subtilis*, *B. tequilensis*, and *B. vallismortis* have been designated as "generally recognized as safe (GRAS)" for human consumption (Lefevre et al., 2017). They have been claimed to be an excellent source of diverse secondary metabolites harboring biopreservative potential, like bacteriocins, lipopeptides, polyketides, siderophores, bacteriocin-like inhibitory substances, and non-ribosomally synthesized peptides (Harwood, Mouillon, Pohl, & Arnau, 2018). Bacteriocins are ribosomal antimicrobial peptides biosynthesized by bacteria for self-defense against the growth of closely related species, and curtain foodborne pathogens through pore formation on the cell surface and interference in cell wall synthesis (Harwood et al., 2018). Bacteriocins produced by *Bacillus* species have been considered as appropriate alternative to chemical preservatives because they represent diverse structural diversity, selectivity towards targeted pathogens owing to both available broad-spectrum and narrow-spectrum activity, stability in a wide conditions of temperature and pH, low allergenic potential, activity at low concentrations, and proteinaceous nature thereby readily being degraded in the gastrointestinal tract (Harwood et al., 2018; Yi, Luo, & Lü, 2018). Currently, only nisin, a pentacyclic bacteriocin produced by certain strains of *Lactococcus lactis* subsp. *lactis*, is commercially available, and legally used as a food additive in Thailand (Notification of Ministry of Public Health, 2018), despite a number of bacteriocins extensively purified and characterized to date.

Selection of bacteriocin-producing species used in food preservation requires close scrutiny in terms of biosafety and beneficial characteristics. Recently, a novel identified strain of *B. velezensis* BUU004 has been confirmed as a promising probiotic and a safe source of biopreservative to prevent foodborne infections of seafood products (Butkhot, Soodsawaeng, Boonthai, Vuthiphandchai, & Nimrat, 2020; Butkhot, Soodsawaeng, Vuthiphandchai, & Nimrat, 2019b). In laboratory study, *B. velezensis* BUU004 has shown strong antibacterial potential against seafood spoilage and pathogenic bacteria, namely *B. coagulans*, *E. coli*, *E. coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* Typhimurium, susceptibility to antibiotics commonly used in human and veterinary clinical therapy, and robust survivability under gastric and bile conditions. The authors also described its non-pathogenic characteristic indicated by low cytotoxicity, and the absence of hemolytic activity and virulence-associated genes: hemolysin genes (*hlyI/clo*, *hlyII* and *hlyIII* homolog), enterotoxin genes (*cytK*, *hblACD* and *nheABC*), hemolytic enterotoxin HBL (*hblABCD*), non-hemolytic enterotoxin NHE (*nheABC*) and cereulide (*cesABCD*). In order to determine if the new *Bacillus* strain intended for use as a source of natural preservative in human foods, the objectives of this study were to investigate antibacterial activity of semi-purified solution containing bacteriocin from *B. velezensis* BUU004 (SPS-BV) against foodborne pathogenic *B. cereus* under *in vitro* environment and food spoilage bacteria in RTE dried, seasoned and crushed squids during storage, and evaluate its effect on physical and chemical qualities (pH, water activity, moisture content, and NaCl content) of the RTE dried squids. In addition, RTE dried seafood products can become potential vehicles of such hazards as pathogen contamination during their

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journeys through all stages of the preparation. Consequently, bacteriological quality across multiple steps related to preparation of the RTE dried squids was also evaluated**.**

2. Materials and Methods

2.1 Bacteriological evaluation across a series of preparation of dried seasoned and crushed squids

2.1.1 Dried squid preparation

Preparation of dried, seasoned and crushed squids was conducted in a small household facility located in a fishing village in Chon Buri province to simulate a real situation of the dried squid production in Thailand (Nimrat, Soodsawaeng, Rattanamangkalanon, Boonthai, & Vuthiphandchai, 2021). In brief, fresh splendid squids (*Loligo duvauceli*) were caught about 10-km offshore from Bang Sean Beach and its vicinity in the Gulf of Thailand by local fishermen. The preparation was initiated by cutting longitudinally in the center of the abdomen of medium-sized squids using a sharp knife, and removing the head, tentacles, skin, eyes, soft shell, beak and internal organs, and then three-time rinsing with tap water (Figure 1a). The 5-kg squids were marinated in homemade Thai seasoning sauce consisting of chili sauce (1.5 L), sugar (500 g), chili pepper powder (80 g), chopped garlic (10 g), and vinegar (50 mL) for 1 h (Figure 1b). Smooth squids were placed on a steel wire grate (Figure 1c), and sun-dried from 8 am to 4 pm for two successive days (Figure 1d). The semi-dried squids were individually crushed using a rolling machine after which they were grilled on an electric grill with periodically flipping over for 2 min (Figure 1e). The crushed squids were sun-dried again for 1 day prior to package in a plastic bag and storage at room temperature (Figure 1f). Sampling was taken in rinsed squid, marinated squid, first sun-dried squid, grilled squid, crushed squid, and second sun-dried squid to assess bacterial quality.

2.1.2 Microbiological analysis

Bacteriological evaluation across preparation steps of dried, seasoned and crushed squids was performed following a method recommended by US Food Drug Administration [FDA] (1998) with some modifications. A portion (50 g) of samples was homogenized with Butterfield's phosphate-buffered water (450 mL) using a stomacher for 2 min, and successive 10-fold dilution was made in the same buffered solution. A minute volume (0.1 mL) of each dilution was seeded onto Plate Count Agar (Becton BD, Sparks, MD, USA). After incubation at 35 ± 2ºC for 24 h, bacterial colonies were enumerated and calculated as colony forming unit (CFU) of total viable count (TVC) per g of sample. Single colony of isolated bacteria with distinct morphological differences was selected and purified on Trypticase Soy Agar (TSA; Becton BD, Sparks, MD, USA). Identification of bacterial species was made following a classical protocol (Winn et al., 2006) and API test kits (bioMerieux, Marcy I' Etoile, France). All measurements were carried out in triplicate.

2.2 Bacteriocin produced by *B. velezensis* BUU004 and its inhibitory spectrum against *B. cereus*

B. velezensis BUU004 strain was isolated from black tiger shrimp (*Penaeus monodon*) pond sediment and used as probiotic in shrimp culture (Butkhot et al., 2019a). The strain was routinely grown aerobically on a TSA plate at 30ºC. The fresh overnight strain was seeded in Trypticase Soy Broth (TSB; Becton BD, Sparks, MD, USA) at 30ºC, 200 rpm for 18 h. Cell-free supernatant was harvested by centrifugation at 8,000*g* and 4ºC for 10 min, and then partially purified using ammonium sulfate precipitation method (An et al., 2015). The supernatant was saturated with 80% ammonium sulphate subsequently with a constant stirring at 4°C overnight. The precipitates were harvested by centrifugation at 10,000*g*, 4ºC for 30 min and the pellets were dissolved in 50 mM sodium phosphate buffer (pH 7.0) prior to dialysis against a dialysis membrane (1 kDa cutoff, Spectrum Laboratory, Los Angeles, CA, USA) at 4ºC overnight. The obtained SPS-BV was filtered through a 0.45 µm filter (Sartorius, Bedford, MA, USA). The SPS-BV contained bacteriocin displaying proteinaceous nature with molecular weight of ca. 5.75 kDa as evaluated by Tricine SDS-PAGE analysis (Butkhot et al., 2019b). Bacteriocin activity of the SPS-BV was assessed against indicator *B. cereus* TISTR 687 using an agar well diffusion method. Two-fold serial dilution of the SPS-BV was prepared, and the activity was calculated as arbitrary units (AU) per mL on a basis of the formula: $2^n/V$ x 1,000 (µl), where n is the reciprocal of the highest dilution at which bacteriocin produces a clear zone of growth inhibition of the indicator strain, and V is volume of each dilution added into the wells (Butkhot et al.,

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2019a). The SPS-BV solution (800 AU/mL) was prepared and stored at -80°C for further use.

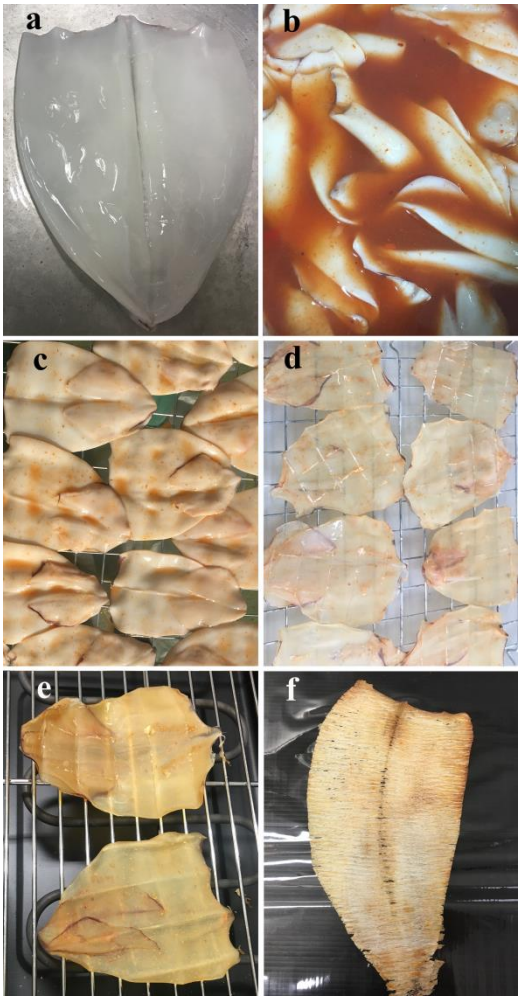

Figure 1. Dried, seasoned and crushed squids during multistep related to preparation processes: (a) rinsed squids, (b) marinated squids in seasoning sauce, (c) squids on a steel wire grate, (d) first sun-dried squids, (e) squids grilled on an electric grill, and (f) dried, seasoned, and crushed squids.

2.2.1 Inhibitory activity against *B. cereus*

Antibacterial activity was investigated using an agar well diffusion assay against *B. cereus* TISTR 687 employed as an indicator (Abdhul et al., 2015). *B. cereus* suspension was prepared at a cell density of 10^8 CFU/mL using the 0.5 McFarland turbidity standard, and then swabbed on the surface of Mueller Hinton agar (Becton BD, Sparks, MD, USA) plates. Thereafter, 6-mm diameter wells were punched by a sterile cork borer. A hundred microliters of the SPS-BV solution (800 AU/mL) was loaded in the wells. All petri dishes were incubated at 35°C for 24 h and diameters of inhibition zone were measured. The experiment was carried out in triplicates.

2.2.2 Scanning electron microscopy

Morphological changes of *B. cereus* treated with the SPS-BV solution were visualized using scanning electron microscopy (SEM). Pathogenic *B. cereus* TISTR 687 was subcultured in TSB 35°C for 18 h to produce the logarithmic-phased cells, and then adjusted to a density of 10^8 CFU/mL using the 0.5 McFarland standard. The adjusted cell suspension was incubated with the SPS-BV solution (800 AU/mL) at 35±1ºC for 20 h. The cell suspension incubated in sterile TSB without any additives was considered as a negative control. The untreated and treated cells of *B. cereus* were centrifuged at 8,000*g*, 4°C for 15 min. Afterwards, the obtained pellets were washed twice with phosphate buffer solution (pH 7.2) and prepared for morphological observations following a conventional protocol (Ullah et al., 2017). Photomicrographs of the cell damages were visualized using a scanning electron microscope (SEM; LEO 1450 VP, ZEISS, Oberkochen, Germany) equipped with an SEM User Interface, LEO-32 software. Bactericidal potential of the SPS-BV solution was determined by spread-plating a portion of the SPS-BV-treated cells on TSA plates, and comparing with untreated cells.

2.3 Antibacterial potential of the SPS-BV in dried, seasoned and crushed squids

2.3.1 Effect of the SPS-BV on food-spoilage bacteria

Nisin consisting of 2.5% active nisin was purchased from Sigma-Aldrich Chemical Co, Darmstadt, Germany. Nisin powder (100 mg) was dissolved in 0.02 N HCl solution to produce nisin solution at a concentration of 10^3 IU/mL. The solution was sterilized by filtering through a 0.45 µm syringe filter, and stored at -20°C for further use (Soodsawaeng, Butkhot, Boonthai, Vuthiphandchai, & Nimrat, 2021). Lactic acid solution containing 80% lactic acid purchased from Sigma-Aldrich, St. Louis, MO, USA was used for preparation of 5 mM lactic acid solution.

The SPS-BV with strong inhibitory activity towards foodborne pathogenic *B. cereus* was employed to control the growth of food-spoilage bacteria in post-prepared dried, seasoned and crushed squids in comparison with commercial preservatives including lactic acid and nisin. First,

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the dried, seasoned and crushed squids were cut into a 2 x 2 cm piece using sterile scissors. The pieces of squid samples were divided into 4 groups including addition of 1) sterile distilled water (control), 2) 5-mM lactic acid, 3) nisin solution (10^3 IU/mL) and 4) the SPS-BV (800 AU/mL). For each treatment, the tested additive (0.1 mL) was slowly introduced onto whole surface of a square piece of the squid sample using an autopipette to allow maximum absorption into the food matrix, and subsequently air-dried for 15 min in a biosafety cabinet (Soodsawaeng et al., 2021). The additive-treated squids were stored separately in a sterile plastic bag according to their treatments. During storage at room temperature (approximately 25°C), the squids were sampled at 15 min and 1, 7, 14, 21 and 28 days of storage for TVC enumeration as aforementioned method.

2.3.2 Effect of the SPS-BV on physical and chemical properties

Changes in physical and chemical qualities, e.g. pH, NaCl content, moisture content and water activity (a_w) of the SPS-BV-treated squids, nisin-treated squids, and lactic-acid-treated squids were evaluated in comparison with the control. Ten grams of the dried squids were homogenized with distilled water (100 mL) in a stomacher bag. pH values were measured using a regular calibrated pH meter (Metrohm; 913 pH meter, Herisau, Switzerland; Karastogianni, Girousi, & Sotiropoulos, 2016). Dried squid samples (10 g) were finely ground using a blender and placed into an aluminium tray. Then, water activity (a_w) was estimated using a water activity meter (Novasina AG; MS1 Set-aw, Lachen, Switzerland; AOAC, 2000) and moisture content was measured using a moisture meter (Sartorius; MA150, Weender Landstraße 94-108 37075, Goettingen, Germany; AOAC, 2000). NaCl content in the dried squids was quantified using a Volhard method (AOAC, 2000). All measurements were conducted in triplicate.

2.4 Statistical analysis

All data are present as mean±standard deviation. Normality of the data was tested and transformation was made when needed. The data were subjected to a two-way analysis of variance (ANOVA) to determine the difference of measured variables. Significant differences were compared using the post-hoc Tukey's test at the level of $p<0.05$. Statistical analyzes were performed using Minitab version 18.1.0.

3. Results and Discussion

3.1 Bacteriological quality during various steps related to production of dried, seasoned, and crushed squids

Across a series of the preparation, freshly rinsed squids contained low TVC of 1.10±0.00 x 10^3 CFU/g, and then TVC significantly ($p<0.05$) increased to 3.60±1.41 x 10^3 CFU/g in the squids marinated in seasoning sauce. TVC in the squids significantly ($p<0.05$) decreased again to 1.80±0.00 x 10^3 CFU/g after first sun-drying. Afterwards, low TVC in the squids ranging from 1.00±0.00 to 4.00±0.00 x 10^2 CFU/g was observed during the phases between grilling and second sun-drying (Figure 2).

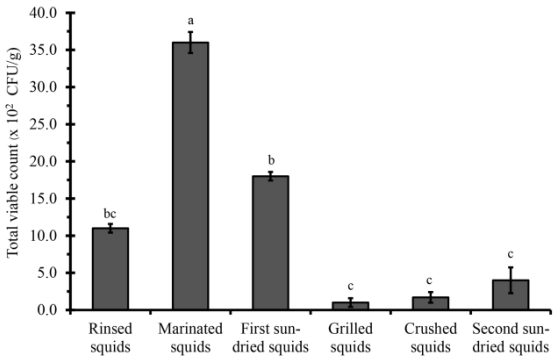

Figure. 2 Bacterial quality across a multistep related to production of dried, seasoned and crushed squids. Letters on bars indicate significant difference ($p<0.05$).

Bacterial composition of the squids changed across a multistep of preparation. In rinsed squids, two species of Gram-positive cocci were isolated including *Kocuria palustris* (86.4%) and *K. varians* (13.6%; Figure 3a). In marinated squids, bacterial component was changed to spore-forming *Bacillus* species, e.g. *B. circulans* (46.1%), *B. coagulans* (35.8%), and *B. acidocaldarius* (18.1%; Figure 3b). Thereafter, *B. acidocaldarius* remained predominant (53.9%) in first sun-dried squids with the presence of two staphylococci species, namely *Staphylococcus saprophyticus* subsp. *bovis* (27.8%), and *S. pasteuri* (18.3%; Figure 3c). Two *Bacillus* species belonging to *B. brevis* (50%), and *B. marinus* (50%) were predominantly isolated from grilled squids (Figure 3d). Then, crushed squids appeared to be dominated by *B. coagulans* (58.8%) and to a lesser extent by *B. Marinus* (20.6%), and *S.*

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saprophyticus subsp. *Bovis* (20.6%; Figure 3e). Lastly, only *B. megaterium* was recovered from second sun-dried squids (Figure 3f).

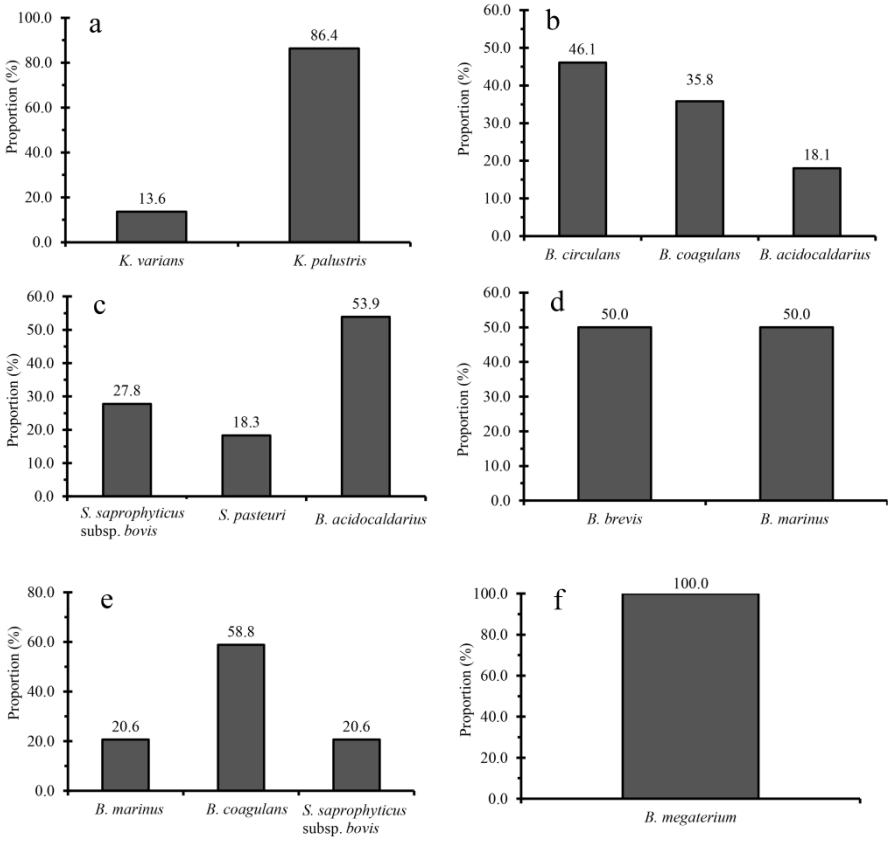

Figure. 3 Composition and abundance of viable bacteria isolated from subsequent phases of the dried squid preparation: (a) rinsed squids, (b) marinated squids, (c) first sun-dried squids, (d) grilled squids, (e) crushed squids, and (f) second sun-dried squids.

A growing demand of traditional RTE seafood products in Thailand has necessitated larger production within the shortest possible time that has placed them at uncertain biosafety status due to a high risk of pathogen contamination. In Thailand, deteriorated bacteriological quality of traditional seafood products has been considered as an important issue in food safety during the past decade (Nimrat et al., 2021). Bacteriological analysis conducted by Butkhot et al. (2019a) has revealed that the biosafety quality of traditional dried seafood-based products in Thailand is relatively poor, indicating a health hazard caused by foodborne diseases. It is widely known that such products can be vehicles of harmful germs causing foodborne illnesses at any stages of preparation. In general, foodborne pathogenic and spoilage bacteria can be introduced into foods at any point of time during the farm-to-fork chain starting from i) primary production (in the farm/sea where animals are raised or caught), ii) preparation processes, iii) transportation, iv) storage, v) distribution, and vi) even the final stage in the consumers' kitchen (Bintsis, 2018). Bacteriological analysis across multiple steps of the food production chain would assist in identifying some potential sources of contamination together with implementation of an effective measure to reduce the health hazard risks of consumers. In the present study, bacterial contamination existed across a series of the preparation of dried, seasoned and crushed squids indicated by the presence of TVC in the ranges of 10^2 – 10^3 CFU/g with abundance of Gram-positive cocci in the genera of *Kocuria* and *Staphylococcus*, and Gram-positive spore-forming *Bacillus* as predominant species. The results were consistent to the recent study conducted by Nimrat et al. (2021). Low TVC of 10^2 CFU/g was observed along a

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multistep production of dried, seasoned and crushed squid with the presence of *Bacillus*, *Staphylococcus*, and *Micrococcus* as main contaminant bacteria. Despite similar types of processed squids, bacterial composition is comparatively different. In general, bacterial abundance in food products is known to vary largely depending on various factors, e.g. raw material used, physical and chemical properties (like a_w, moisture content, pH), storage environment, and food additives and preservatives used. The present study points out that poor hygienic practices still exist during preparation and production processes of traditional dried seafood products in Chon Buri province. In this study, in the post-rinsed phase of the preparation chain, main bacterial compositions of the rinsed squids were *K. palustris* and *K. varians.* Due to their environmental origin, water may be a source of bacterial contamination in the squids (Khan, Knapp, & Beattie, 2016). Municipal water is commonly used in multiple phases of the preparation chain of dried, seasoned and crushed squids in Thailand. In addition, poor personnel hygiene and inadequate handling may increase the transference possibility of *Kocuria* species from worker's hands to the squids because they are indigenous flora on skins, mucous membranes, and oral cavities of humans (Kandi et al., 2016). Our results also revealed that the most common source of spoilage bacterial contamination was brown seasoning sauce used for squid marination supported by the highest TVC number, and bacterial composition shifted from *Kocuria* to *Bacillus* species. In accordance with Nimrat et al. (2021), predominant bacteria isolated from dried, seasoned and crushed squid marinated in brown seasoning sauce were *Staphylococcus* and *Bacillus* species. This can be speculated that *Bacillus* contamination may be linked to spices and condiments, such as chili pepper powder and garlic used as ingredients in the seasoning sauce. Edible spices and dried herbs are usually susceptible to microbial contamination, especially spoilage bacteria belonging to *Bacillus* genera (Sagoo et al., 2009). Antibacterial potential of spices and other additives in the seasoning sauce may also play a role in inhibiting the growth of *Kocuria* present in the preceding phase of preparation (Curtis, Noll, Störmann, & Slusarenko, 2004). As a consequence, brown seasoning sauce should be supplemented with natural preservatives to reduce bacterial contamination, especially spoilage *Bacillus* species. In the next phase of preparation, the bacterial component was changed again to *B. acidocaldarius* as predominant species with a lesser extent staphylococci species in 1st sun-dried squids. In Thailand, traditional dried seafood products are commonly produced in small factories or household facilities with rudimentary hygienic practices, thereby having a free access of pathogens, flies, insects, rodents, dust, soil, and hazardous chemicals and pollutants (Butkhot et al., 2019a). This may account for such a phenomenon. The presence of *S. saprophyticus* subsp. *bovis* and *S. pasteuri* may also reflect a poor handling manner due to their normal flora on skins, anterior nares and ear canals of humans (Becker, Heilmann, & Peters, 2014). In subsequent stages of preparation, *Bacillus* species become the most frequently isolated bacteria with approximately 80-100% of total bacterial isolates recovered from grilled squids, crushed squids, and second sun-dried squids. Of all bacteria isolated, *Kocuria* spp. has occasionally been a cause of infection usually in patients with immunosuppression including urinary tract infections, cholecystitis, catheter-associated bacteremia, dacryocystitis, canaliculitis, keratitis, peritonitis, brain abscess and meningitis (Kandi et al., 2016). Although heat-stable toxin producing strains of *Bacillus* species, especially *B. cereus*, and *B. subtilis* have been reported as cause of food poisoning linked to consumption of contaminated foods (Logan, 2011), there have been no reports of *Bacillus* species isolated from the squid samples in the present study being associated with foodborne illnesses. However, due to resistance characteristics of the endospores to heat, radiation, preservative, and desiccation, they may survive and multiply to high levels when water becomes available during dried seafood products being stored at room temperature for sale. This can create troublesome problems in commercial dried seafood as changes in textures, production of off-flavors, food defects and organoleptic unacceptability of consumers resulting in a significant financial loss.

In order to secure biosafety from a farm to fork cycle of the traditional dried squid products in Thailand, more stringent measures for hygiene at production facilities are required. The introduction of the microbiological guidance, e.g. Good Hygiene Practices (GHPs), Good Manufacturing Practices (GMPs) and Hazard Analysis Critical Control Point (HACCP) programs in place with the establishment of in-process controls to ensure product integrity,

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rather than reliance on end-product testing for compliance with specifications has been a significant advance in production of safer food products and prevention of outgrowth of pathogenic and spoilage microorganisms. Education programs based on basic principles for microbiological food safety of food handlers can be a straightforward strategy to create biosafety awareness and help to prevent foodborne diseases. Moreover, controlling post-prepared contamination may be one of the key aspects of food safety. In addition to good hygiene practices, biopreservative-based strategy, in particular bacteriocins supplementation has received tremendous attention for controlling the growth of pathogen and spoilage bacteria in post-prepared products to minimize the risk of foodborne illnesses.

3.2 Inhibitory potential against pathogenic *B. cereus* of the SPS-BV

The SPS-BV exhibited strong antibacterial activity towards foodborne pathogenic *B. cereus* with an inhibition zone of 14.0±0.2 mm (Figure 4). It had bactericidal activity observed by no growth of the SPS-BV-treated pathogen on the TSA plate whereas the untreated cells grew well on the medium (data not shown). Its mode of action against *B. cereus* was evaluated using SEM analysis. Normal intact cell walls were observed in untreated cells of *B. cereus* (Figure 4a). Conversely, the SPS-BV severely destroyed the cellular structure of *B. cereus* cells evident from pore formation along with extremely flattened cells (Figure 4b). This may be indicative of intracellular fluid efflux.

B. cereus can easily contaminate dried seafood products (Butkhot et al., 2019a) and causes two types of gastrointestinal diseases: diarrhea and emesis (Huang, Flint, & Palmer, 2020). It is difficult to eliminate *B. cereus* contamination in food products due to its heat tolerance of the endospores and easy growth of the vegetative cells at a broad range of temperatures. In the present study, the SPS-BV exhibited antibacterial potential against food poisoning strain of *B. cereus*. The results were corroborated with a study reported by Perumal, Yao, Kim, Kim, and Kim (2019). Culture supernatant containing a bacteriocin BacBS2 produced by *B. velezensis* BS2 isolated from Korean fermented seafood showed strong inhibitory spectrum towards various species of pathogenic Gram-positive and negative bacteria, especially *B. cereus* and *Listeria monocytogenes* in laboratory media. Similarly, Lee and Chang (2017) claimed that *B. subtilis* SN7 strain produced a novel bacteriocin, namely mejucin, with high antibacterial activity against various pathogens, especially *B. cereus*. Then, the SN7 strain was used as a starter culture in chongkukjang, a Korean fermented soybean product, and exhibited a complete growth inhibition of experimentally inoculated *B. cereus* cells during fermentation and 6-month refrigerated storage. SEM analysis revealed that the SPS-BV had bactericidal activity against *B. cereus* through cell lysis supported by pore formation along with severe deconstruction of the cell structure. It seems that mode of action of the SPS-BV in this study may be related to a fluidifying effect on the cellular structure that facilitates depolarization, disintegration of the protective outer membrane, increased membrane permeability, change in the proton motive force, formation of transient pores, efflux of intracellular fluids, and eventually cell death (Malanovic & Lohner, 2016).

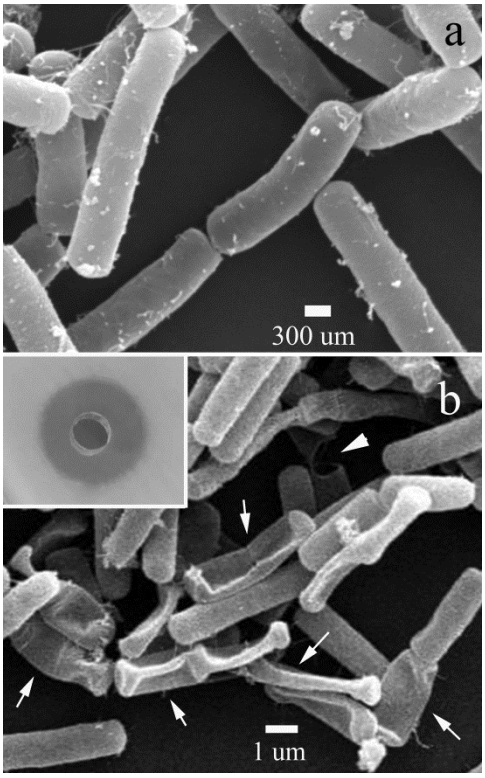

Figure. 4 SEM microphotographs of (a) untreated *Bacillus cereus* representing regular and intact cell walls, and (b) the-SPS-BV-treated cells representing pore formation (➤) and ghost cells with cytoplasmic material completely lost (↑) with inset showing inhibitory activity against *B. cereus* of the SPS-BV based on an agar well diffusion technique.

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The results confirmed antibacterial potential of the SPS-BV for controlling the growth of pathogenic and spoilage bacteria in food products.

3.3 Effect of the SPS-BV on food-spoilage bacteria, and physical and chemical properties of dried, seasoned, and crushed squids

3.3.1 Effect on food-spoilage bacteria in dried, seasoned and crushed squids

During 28-day storage at room temperature, TVC in the untreated squids were in the ranges of 1.75±0.50 - 6.00±1.63 x 10^2 CFU/g (Figure 5). Addition of the SPS-BV was as effective as nisin for controlling the growth of food-spoilage bacteria. TVC in the SPS-BV-treated squids and nisin-treated squids remained comparatively stable ranging from undetectable level to 2.00±0.82 x 10^2 CFU/g, which were significantly (p<0.05) lower than those of the control. Lactic acid could control the growth of spoilage bacteria by 21 days of storage, and thereafter lactic acid-treated dried squids exhibited a significant (p < 0.05) increase in

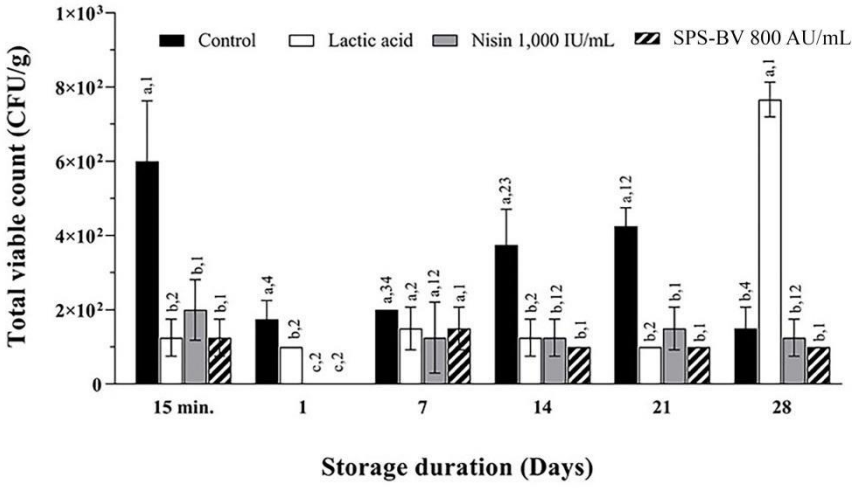

Figure. 5 Effect of the tested additives on food-spoilage bacteria in dried, seasoned, and crushed squids during storage at room temperature for 28 days. Means with superscript letters at each sampling period indicate significant difference (p < 0.05) among treatments. Means with superscript numbers in each treatment indicate significant difference (p < 0.05) over time.

TVC, compared to those of the SPS-BV-treated squids and nisin-treated squids at day 28 of storage (Figure 5). Our results indicated that supplementation of the SPS-BV was as effective as commercial nisin for controlling food spoilage bacteria in dried, seasoned and crushed squids during storage at room temperature. Similar results were reported by Fangio and Fritz (2014). Crude extract of a bacteriocin-like substance produced by a harmless strain of *B. cereus* P9 was administrated in food samples. As a result, it showed a strong antibacterial activity observed by significant reductions in mesophilic and psychrotrophic aerobic bacteria counts in fresh beef and *B. cereus* counts in lettuce (*Lactuca sativa* L.) under refrigerated storage. Tumbarski et al. (2020) also studied the potential use of a novel bacteriocin from *B. methylotrophicus* strain BM47 for biopreservation and improvement of the storage life of fresh tomato (*Solanum lycopersicum* L.) juice. The bacteriocin produced by *B. methylotrophicus* strain BM47 retained low TVC population in fresh tomato juice, especially in combination with pasteurization, compared to the control group during 24-day chilled storage. Reduced TVC population in the RTE dried squids in the present study is possibly expected due to the SPS-BV activity through cell lysis as mentioned previously. Lactic acid has been classified as GRAS by the U.S. Food and Drug Administration. It widely used as a promising alternative of food additive for inhibition of pathogenic and spoilage bacteria in human products due to its antimicrobial activity, flavor enhancer, antioxidant potential, prevention of lipid oxidation by decreasing the pro-oxidative effect of NaCl (Paelinck & Szczepaniak, 2005). In the present

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study, lactic acid demonstrated an immediate antibacterial effect, and prolonged period of bacteriostasis with low TVC population for up to 21-day storage, but thereafter, its inhibitory activity seemed significantly reduced as supported by a pronounced increase in TVC population at day 28 of storage. The results were in contrast to the previous report by Greer and Dilts (1995). Pork with fat tissue was immersed in a 3% lactic acid solution at 55°C. Lactic acid immediately eliminated spoilage bacteria on the pork to below detectable level and no growth of artificially-inoculated meat-spoilage bacteria, e.g. *Pseudomonas fragi* and *Brochothrix thermosphacta* were observed throughout 15-day storage at 4°C. Conversely, the spoilage bacterial numbers were approximately 8 log cycle in water-treated pork. Discrepancies on the ability of lactic acid for controlling the growth of spoilage bacteria on food products could be related to food types, treatment conditions, and sensitivity of indigenous flora to the organic acids. Inactivation of lactic acid activity is possibly associated, in part with the fact that lactic acid concentration progressively decreases during storage to a level at which inhibitory potential against spoilage bacterial cells is faded out in food matrix due to its high reactivity of the two adjacent functional groups: acid and alcohol, in the molecules (Komesu et al., 2017).

3.3.2 Effect on physical and chemical properties of dried, seasoned and crushed squids

Lactic acid, nisin, and the SPS-BV influence physical and chemical changes of dried, seasoned and crushed squids in a different way. Addition of either nisin or the SPS-BV resulted in a significant ($p < 0.05$) increase in pH values (5.79±0.03 and 5.81±0.04) at 15-min post-exposure, and afterwards, similar pH values were recorded in nisin-treated, SPS-BV-treated squids, and the control during 28-day storage, except at 21-day storage of nisin-treated squid (Figure 6a). Moisture contents of nisin-treated, and SPS-BV-treated squids ranged from 13.2±1.4 - 20.8±1.0%, and 13.2±0.5 - 22.0±0.3%, respectively, which were significantly lower than that of the control (13.5±1.0 - 26.5±0.0%) throughout 28-day storage, except at day 14 and 21 of storage (Figure 6b). Similar to moisture content, a_w values of the dried squids significantly ($p < 0.05$) decreased in the ranges of 0.571±0.001 - 0.739±0.004 and 0.568±0.003 - 0.707±0.000 following addition of nisin and the SPS-BV, respectively in comparison with the control (0.570±0.004 - 0.738±0.003) during 28-day storage, except at 14-day post-storage (Figure 6c). All tested additives had no effect on NaCl content in the dried squid samples during storage (Figure 6d).

Physical and chemical quality is an important parameter to indicate the food quality and its change can affect sensorial quality and consumer acceptability. In this study, significant reductions of moisture content and a_w were observed simultaneously with no effects on pH and NaCl content following addition of the SPS-BV in the RTE dried squids. Generally, a_w is related to water present in food in free form necessary for microbial growths, chemical-biochemical reactions, rates of deteriorative reactions, and physiochemical properties of food products (Fontana, 2000). a_w of the SPS-BV-treated squids significantly decreased in comparison with the control during storage. Similarly, Arief, Jenie, Suryati, Ayuningtyas, and Fuziawan (2012) reported that addition of 0.3% bacteriocin solution from *Lactobacillus plantarum* 2C12 caused a reduced a_w in beef ball while a_w of the untreated beef ball obviously increased during 6-day refrigerated storage. In fact, a decrease in a_w is a desirable environment to inhibit the growth of spoilage bacteria in dried food products. In this context, the significant reduction in a_w of the SPS-BV-treated squids may account for such decrease in TVC population in the present study. Relationship between the SPS-BV addition and a change in a_w is currently unknown in the dried squid samples. A significant reduction in a_w of the SPS-BV-treated squids may be explained by an osmotic dehydration process as observed in several products following

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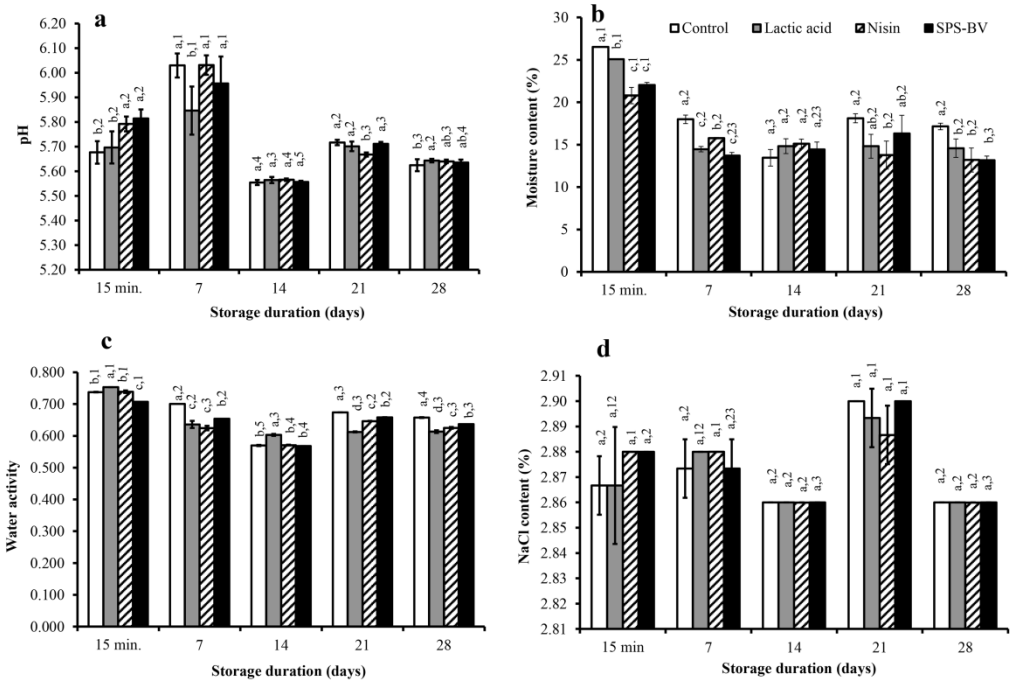

Figure. 6 Physical and chemical changes: (a) pH, (b) moisture content, (c) water activity, and (d) NaCl content of dried, seasoned and crushed squids following addition of the tested additives during storage for 28 days. Means with superscript letters indicate significant difference ($p < 0.05$) among treatments. Means with superscript numbers indicate significant difference ($p < 0.05$) over time.

addition of solutes into foods such as NaCl, sucrose, glucose, and inverted sugar. The mechanisms involve a water-soluble solute flow into the food matrix and an outflow of free water from the product into the environment resulting in increased diffusion of active antibacterial agents for improving biosafety quality (Erkmen & Bozoglu, 2016). Despite strong antibacterial activity, a_w of the SPS-BV-treated squids was over allowable limit at 0.6 of dried RTE squids distributed in Thailand (Thai Industrial Standards Institute, 2010). This indicates a need for an effective alternative strategy for administration of the SPS-BV in RTE dried squids while maintaining its antibacterial activity. Edible films derived from natural products are such examples of technological application, which can be a promising method serving as carriers and providing a controlled release of antimicrobials over an extended period of time without deteriorative effect on physical and chemical quality of foods. Moisture content influences food characteristics, e.g. physical appearance, texture, taste, shelf-life, and even resistance to spoilage caused by bacterial activity. Even though moisture content of the SPS-BV treated squids obviously reduced during storage in comparison with the control, there is no regulatory standard for RTE squid products imposed by the food administration agency of Thailand. A change in pH of foods may affect its color and sensorial quality. Such increase in pH reflects meat spoilage due to protein decomposition and the formation of alkaline compounds. However, pH of the SPS-BV-treated squids did not significantly differ with the control during storage, suggesting a protective activity against proteolytic reactions of the additive.

Although nisin is accepted as an effective and safe antibacterial peptide with FDA-approved and GRAS status and commonly used as food preservative in Thailand and other countries, such limitation of nisin usage in commercial foods is compromised by its expensiveness, lack of inhibitory actions against Gram-negative bacteria, and development of nisin-resistant pathogenic strains (Zhou, Fang, Tian, & Lu, 2014). Our results confirm an equal antibacterial activity of nisin and the SPS-BV for controlling the growth of food spoilage bacteria in RTE dried squids. This suggests that application of the SPS-BV can meet demand for natural preservatives and provide financial benefits by reducing the costs of treatment in RTE dried seafood products. However, an additional study

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focused on organoleptic quality is required to ensure the consumer acceptability of dried, seasoned and crushed squid in terms of sensorial properties.

4. Conclusions

Across a series of the preparation, TVC populations between rinse and 2nd sun-dry phases were in the ranges of 10^2 - 10^3 CFU/g. The most common source of spoilage bacteria contamination was brown seasoning sauce used for squid marination supported by the highest TVC population and diversity. Main contaminant bacteria isolated from prepared dried squids were *Bacillus*, *Kocuria* and *Staphylococcus.* The SPS-BV was as effective as commercial nisin in terms of controlling food spoilage bacteria in dried, seasoned and crushed squids during storage without effects on pH and NaCl content. However, significant reductions in moisture content and a_w were produced following addition of the SPS-BV in the dried squids. Mode of mechanisms of the SPS-BV against spoilage and pathogenic bacteria was through cell lysis supported by pore formation along with severe deconstruction of the cell structure. The results suggest application potential of the SPS-BV as biopreservative in RTE dried seafood products.

Acknowledgements

This study was sponsored by the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0109/2556) to S. Nimrat and P. Soodsawaeng, and the Research Grant of Burapha University through National Research Council of Thailand (Grant no. 66/2558). We thank Biological Science Program, and Department of Microbiology, Faculty of Science, Burapha University, Thailand for providing experimental equipment and facilities.

Conflict of Interest

No conflict of interest declared.

ORCID

Pornpimon Soodsawaeng:
https://orcid.org/0000-0001-7793-7684
Traimat Boonthai:
https://orcid.org/0000-0002-8251-6486
Verapong Vuthiphandchai:
https://orcid.org/0000-0003-3311-4939
Subuntith Nimrat:
https://orcid.org/0000-0001-9327-2408

Ethical Approval

All procedures in this study were in accordance with the ethical standards approved by the Research Unit Committee, Faculty of Science, Burapha University (September 1, 2018).

Burapha University Animal Care and Use Committee

Publication Ethic

The submitted manuscript has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere.

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Mechanical Properties Enhancement of the Cement Mortar by Synthetic Zeolite Polymer Composites

Kisun Chunti[1], Parinya Chakartnarodom[1], Passakorn Sonprasarn[1], Wichit Prakaypan[2], Edward A. Laitila[3], Sureerat Polsilapa[1*]

[1]Department of Materials Engineering, Faculty of Engineering, Kasetsart University, Chatuchak, Bangkok 10900, Thailand
[2]UAC Global Public Company Limited, Chatuchak, Bangkok 10900, Thailand
[3]Department of Materials Science and Engineering, Michigan Technological University, Houghton, MI, 49931, USA
*Corresponding author e-mail: fengsrsn@ku.ac.th

Received: *25 October 2022* / **Revised**: *12 December 2022* / **Accepted**: *19 December 2022*

Abstract

The performance of cement mortar can be improved with additives based on waste by-products. Synthetic zeolite polymer composites (referred as SZPC) produced from the combination of solid waste ashes with a selective acrylic compound was used as a cement mortar additive. The effect of SZPC as an additive on hydration reaction of ordinary Portland cement (referred as OPC) at different amounts of SZPC, from 1-4% of OPC weight, as well as microstructure and mechanical behavior of the cement mortar are determined. The results from the hydration reaction rate test showed that the optimum amount of SZPC as the additive was 2% of OPC weight. Compressive strength and flexural strength of the cement mortar after 1, 7, 14 and 28 days of curing increased, with the largest increases at the early stage. Additions of SZPC, synthesized by a waste by-product, improved mechanical behavior of cement mortars supporting sustainable development and the circular economy.

Keywords: Cement mortar, Zeolite, Pozzolan, Ettringite

1. Introduction

The principles of sustainable development and the circular economy have gathered more attention in recent years. Numerous countries embrace the concepts of efficient energy conservation, greenhouse-gas emission reduction, and efficient commodities improvements (Velenturf & Purnell, 2021). To comply with these concepts, it is necessary to consider how high-performance products can be utilized to lengthen the lifetime of the current products and reduce the manufacturing requirements. In the concrete industry, every ton of manufactured cement generates 0.85 tons of CO_2 emissions. Moreover, 5-7% of worldwide greenhouse gas emissions are produced by the cement production sector (Devi, Lakshmi, & Alakanandana, 2018).

Cement mortar is a cement-based material prepared from ordinary Portland cement (referred as OPC), sand, and water (Parveen Kumar & Radhakrishna, 2015). Commonly, cement mortar is used for adhering construction bricks and blocks, plaster for building interior walls, as well as many other constructions uses. Many have studied improving the performance of cement mortar, for example. Gbekou, Benzarti, Boudenne, Eddhahak and Duc (2022) found that there was a reduction in density and improvement of the thermal properties of Shicement mortar when microencapsulated phase change materials were added. However, the mechanical strength of cement mortar diminished, and porosity also increased. According to Jiang, Li, Liu, He and Hernandez (2022) using recycled concrete powder as a sand replacement for cement mortar decreased the bulk density of the mortar while the mechanical properties were slightly improved.

Another method to improve the mechanical properties is the use of nanomaterials. He et al. (2022)

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found that using graphene as an additive could significantly improve the flexural strength and compressive strength of cement mortar. Shi et al. (2022), added carbon-nanofibers that improved both flexural strength and the modulus of elasticity of the cement mortar significantly. Moreover, Zhang et al. (2021) used nanocomposites to improve the early strength of cement mortar.

Alumino-silicate is a chemical additive that can improve the early strength and reduce the setting time of the concrete due to its pozzolanic property (Zhang & Malhotra, 1995). Synthetic zeolite is an alumino-silicate compound manufactured by a thermal process (Zhao, 2010). Moreover, synthetic zeolite can be synthesized from the waste by-product of AlF_3 production. As a concrete additive, synthetic zeolite can improve the performance of concrete; for example, increased strength and freeze-thaw resistance (Girskas, Skripkiūnas, Šahmenko, & Korjakins, 2016).

To promote the mega trend following the circular economy, the improvement of the properties of the related cement product is inspired to expand the time of consumption by the cement additives, especially, the treated waste additives. Synthetic zeolite polymer composites (referred as SZPC) produced from the combination of solid waste ashes with a selective acrylic compound is used as a cement mortar additive. The influences of SZPC on the mechanical properties and microstructure of the cement mortar are observed.

2. Materials and Methods

2.1 Materials

The raw materials are ordinary Portland cement (OPC), sea sand (particle size -325 mesh), with the additive SZPC. These materials were supplied by Shera Public Company Limited. Chemical composition of the raw materials was determined from pressed powders by x-ray fluorescence spectrometer (XRF, Panalytical-Minipal 4) with results provided in Table 1.

Table 1. Chemical composition of raw materials.

Compound	Sample (wt%)		
	Sand	**OPC**	**SZPC**
SiO_2	97.06	20.01	40.60
Al_2O_3	1.10	6.25	37.44
Na_2O	0.00	0.00	21.05
K2O	0.00	0.30	0.03
CaO	0.24	64.32	0.07
Fe_2O_3	0.00	3.25	0.02
MgO	0.0	1.02	0.00
SO_3	0.00	2.33	0.77
Others	1.35	2.52	0.02

2.2 Hydration reaction rate test

The effect of SZPC on the hydration reaction of OPC is determined from the temperature change due to the heat released from the reaction. The dry mixtures consisting of OPC and SZPC (0 to 4% of OPC weight) were mixed with water to create a slurry using the water-to-cement ratio of 0.3. The slurry was kept in the chamber as shown in Figure 1. The heat generated within the chamber because of the hydration reaction was measured by the thermocouple every 30 s until the temperature inside the chamber was constant. The test was carried out based on the method described in ASTM standard C95/95M. (Pahuswanno, Chakartnarodom, Ineure, & Prakaypan, 2019; Sonprasarn, Chakartnarodom, Ineure, & Prakaypan, 2019).

The hydration reaction rate, represented by the average rate of temperature change from the initial temperature to the maximum temperature, is calculated by (Chakartnarodom, Wanpen, Prakaypan, Laitila, & Kongkajun, 2022; Sonprasarn et al., 2019):

$$r = \frac{T_{max} - T_i}{t} \quad (1)$$

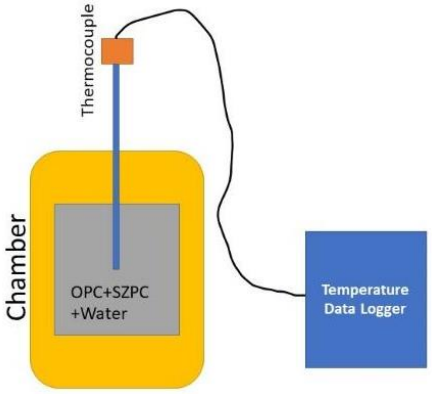

Figure 1. The schematic drawing of the instrument used for hydration reaction rate test.

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2.3 Sample preparation and testing

Cube-shaped samples (5 cm × 5 cm ×5 cm) and rectangle-shaped samples (4 cm × 4 cm × 16 cm) were casted based on the formulas listed in Table 2. The dry mixtures in Table 2 were mixed with water using a water-to-cement ratio of 0.45. A SZPC amount of 2% of OPC weight was determined from the hydration reaction test (section 3.1). After 1, 7, 14 and 28 days of curing, the compressive strength is determined on the cube-shaped samples while the flexural strength is determined on the rectangle-shaped samples. The mechanical testing was carried out based on ASTM standard C90: standard specification for load-bearing concrete masonry unit, using a universal testing machine (UTM, Instron machine 3300). Five samples from the same formulas were used for each test. Additionally, the microstructure of the samples was characterized by a scanning electron microscope (SEM).

Table 2. Formulas for sample preparation.

Formula	OPC (wt%)	Sand (wt%)	SZPC (% of OPC weight)
REF	75	25	-
HSZ2	75	25	2

3. Results and Discussion

3.1 Rate of hydration reaction

The effect of the SZPC concentrations ranging from 1-4% of OPC weight on the hydration reaction rate of OPC are presented in Figure 2 and Table 3. The initial temperature for the test was about 28°C.

Based on the reaction rate calculation, Table 3, the reaction rate is improved by 25% with the addition of SZPC at 2% of OPC weight. This improved reaction rate should be from the pozzolanic reaction between SiO_2 and Al_2O_3 in SZPC with $Ca(OH)_2$, a product from the hydration reaction, which produces calcium silicate hydrate (C-S-H) and calcium aluminosilicates hydrate (C-A-S-H) as shown in equation (2) and (3) (Tran, Lee, Kumar, Kim, & Lee, 2019):

$$2SiO_2 + 3Ca(OH)_2 \rightarrow 3CaO \cdot 2SiO_2 \cdot 3H_2O \quad (2)$$

$$Al_2O_3 + 3Ca(OH)_2 \rightarrow 3CaO \cdot Al_2O_3 \cdot 6H_2O \quad (3)$$

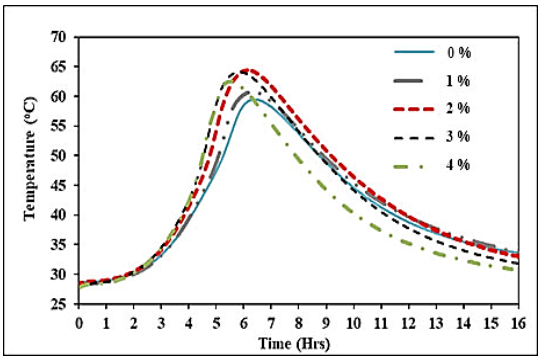

Figure 2. Results from hydration reaction rate test.

Table 3. Hydration temperatures, times, and reaction rates of SZPC on OPC.

SZPC concentration (% of OPC weight)	Max Temp. (°C)	Time (hr:min)	Reaction Rate (°C /min)
0 (Control)	59.0	6:28	0.08
1	60.7	6:11	0.09
2	64.4	6:13	0.10
3	64.1	5:55	0.10
4	62.6	5:33	0.10

Additions of SZPC over 2% of OPC weight does not have any further effect on the reaction rate. According to Marchon and Flatt (2016) and the hydration products, such as C-S-H, will nucleate and grow over the surface of cement particles and other seeding particles. However, thickening of C-S-H layer, and particle impingement during the growth of C-S-H layer could reduce the hydration rate (Chakartnarodom et al., 2022; Zhou, Duan, Tang, Chen, & Hanif, 2019). Therefore, based on this work, the optimum amount of the SZPC is 2% of OPC weight.

3.2 Mechanical properties of the cement mortar samples

The compressive and flexural strengths of the cement mortar samples are shown in Figure 3 and Figure 4 respectively. Obviously, there is strength development from 1 to 28 days of curing for both the REF and HSZ2 samples. However, the SZPC has a noticeable effect on all the mechanical properties of

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HSZ2 samples, especially after 1-day of curing in which the compressive strength and flexural strength of HSZ2 samples are higher than that of REF samples by 68.44% and 27.27% respectively. The significant development of early strength should be from the pozzolanic reaction in the SZPC as described by equation (2) and (3).

The microstructure of the REF sample, Figure 5 (a), is mainly amorphous C-S-H (Chakartnarodom et al., 2022; Harrisson, 2019; Maljaee et al., 2021). Moreover, the REF sample clearly exhibits pores inside its structure, however after adding SZPC as the HSZ2 sample, Figure 5 (b), the replacement of the needle-like crystals is observed by filling those pores. According to Tanasalagul et al. (2019), this crystalline phase is ettringite. According to McCarthy and Dyer (2019), when pozzolan is added to the cement, ettringite will rapidly form and can be observed in the cement for up to 28 days or more. Yu, Qian, Tang, Ji and Fan (2019) determined the formation of the ettringite phase improves the compressive strength of the cement by providing the dense structure and low porosity. Therefore, the decrease in porosity and increase in dense structure of cement mortar due to the presence of ettringite crystals after the addition of SZPC could enhance the compressive and flexural strength of HSZ2 samples.

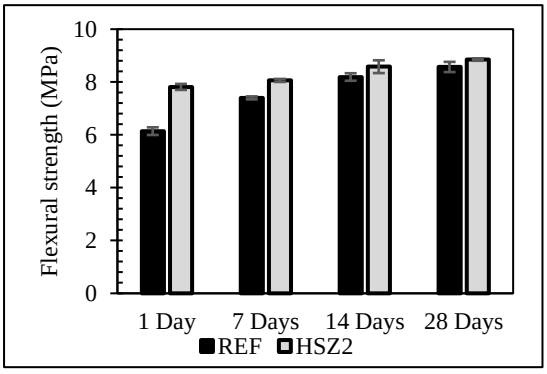


Figure 3. Compressive strength of the samples.

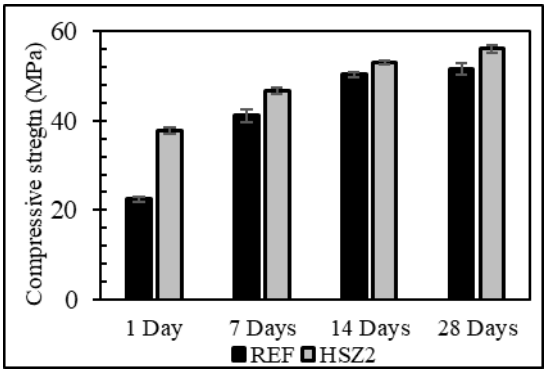
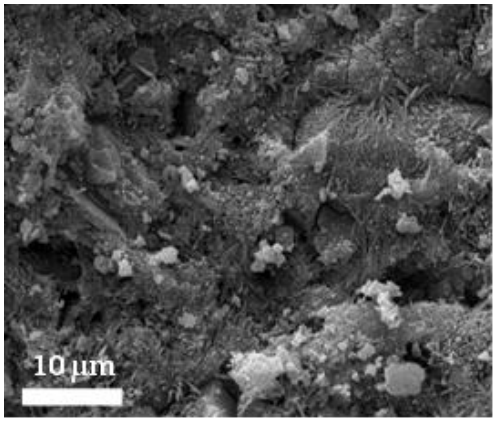
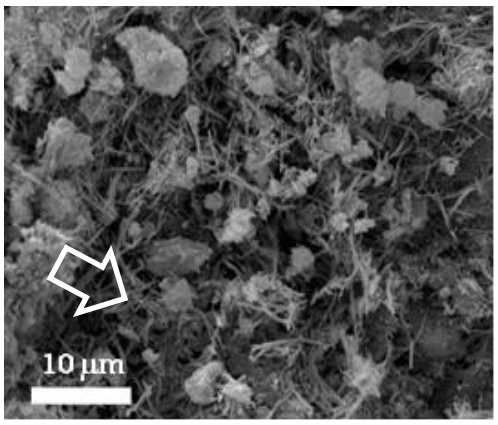


Figure 4. Flexural strength of the samples.



(a)



(b)

Figure 5. Microstructure of the samples after 1 day of curing (a) REF (b) RSZ2.

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4. Conclusions

The influences of synthetic zeolite polymer composites (referred as SZPC) on the mechanical properties of cement mortar were investigated. The SZPC was prepared from the solid waste ashes with a selective acrylic compound. The SZPC affected on the increase in the hydration reaction rate of cement by the pozzolanic reaction. Based on the hydration reaction rate test, the optimum amount of SZPC added to the cement mortar samples was 2 wt% of the weight of ordinary Portland cement. By using SZPC, there is improvement of both compressive and flexural strengths, most prominent during the early curing stages due to the change in microstructure by enhancing the existence of ettringite crystals which was provided the dense structure of cement mortar.

Acknowledgement

This research was funded by ASEAN University Network/Southeast Asia Engineering Education Development Network (AUN/SEED-Net JICA); Department of Materials Engineering, Faculty of Engineering, Kasetsart University; International Collaborative Education Program for Materials Technology, Education, and Research (ICE-MATTER); Shera Public Company Limited; and Sun Cement Process Company Limited.

Conflict of Interest

The authors declare no conflict of interest.

ORCID

Kisun Chunti
https://orcid.org/0000-0002-2555-278X
Parinya Chakartnarodom
https://orcid.org/0000-0002-4873-7285
Passakorn Sonprasarn
https://orcid.org/0000-0002-7271-7727
Wichit Prakaypan
https://orcid.org/0000-0002-4904-1992
Edward A Laitila
https://orcid.org/0000-0003-4868-9305
Sureerat Polsilapa
https://orcid.org/0000-0003-2376-732X

Publication Ethic

The authors declare that this submitted manuscript has not been previously published by or be under review by another print or online journal or source.

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Impact of the Hybrid-Aluminum Additive on the Hydration Kinetics of Portland Cement in Fiber-Reinforced Cement Composites

Juthamat Nithipaiboon[1], Wichit Prakaypan[2], Parinya Chakartnarodom[3], Edward A Laitila[4], Nuntaporn Kongkajun[1*]

[1]Department of Materials and Textile Technology, Faculty of Science and Technology, Thammasat University, Pathum Thani, 12120, Thailand
[2] UAC Global Public Company Limited, Chatuchak, Bangkok, 10900, Thailand
[3]Department of Materials Engineering, Faculty of Engineering, Kasetsart University, Bangkok, 10900, Thailand
[4]Department of Materials Science and Engineering, Michigan Technological University, Houghton, MI, 49931, USA
***Corresponding author e-mail: n-kongkj@tu.ac.th**

Received: *10 October 2022* / **Revised**: *14 December 2022* / **Accepted:** *19 December 2022*

Abstract

A hybrid-aluminum additive (HAA) synthesized from industrial wastes including aluminum dross and flue gas desulfurized (FGD) gypsum was used as an additive for fiber-reinforced cement composites (FRCC). The impact of this additive on hydration kinetics was observed by the temperature change over time for the various HAA mixtures with ordinary Portland cement (OPC), sand, cellulose fibers, polyvinyl alcohol (PVA) fibers, and water, based on the method described in ASTM C186-98. The results showed that the hydration kinetics of OPC in the FRCC was improved by using HAA. In addition, when the amount of HAA was at 3% of the OPC weight, the hydration reaction rate was improved by 41%. The HAA additive acted as an accelerating agent by shortening the setting time and enhancing the temperature of the hydration reaction. This suggests that the cement paste can set faster, reducing the cycle time in FRCC processing. Even though further addition of the HAA increased the reaction rate, the setting time of OPC was too short to form a green sheet for the actual production of FRCC on an industrial scale. In addition, the heat released during the test, representing by the temperature change of the sample, was too high which could have a negative impact on the finished FRCC products.

Keywords: Hydration, Cement, Additive, Circular economy, Recycling, Waste

1. Introduction

The FRCC are construction materials commonly used in the roofing, flooring, wall, and ceiling of a building (Figure 1). Generally, this material exhibits exceptional mechanical properties and durability. The basic raw materials for manufacturing FRCC are ordinary Portland cement (OPC), silica sand, fibers, additives, and water. The mixtures are shaped into the green sheets by manufacturing processes such as filter pressing, Hatschek process, extrusion, mold casting, flow-on process, and roll forming. After molding, the green sheets are cured either by air or an autoclave. Normally, the water-cement ratio in FRCC production can be as high as 0.3, which significantly affects the kinetics of the hydration reaction between OPC and water during the curing stage.

Figure 1. Various FRCC products with different applications available at a hardware store.

Cement additives are materials added to cement-based products to improve their performance and properties such as increasing

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hydration reaction rate and toughness, density, water absorption, and enhancements to the strength after curing. Therefore, an additive that can improve the reaction kinetics would be beneficial. Many researchers have studied the effect of additives on physical properties of cementitious materials (Chakartnarodom et al., 2020; Chakartnarodom Prakaypan, Ineure, Kongkajun, & Chuankrerkkul, 2018; Chakartnarodom, Wanpen, Prakaypan, Laitila, & Kongkajun, 2022; Khorami & Ganjian, 2011; Mohr, Nanko, & Kurtis, 2005; Pahuswanno, Chakartnarodom, Kongkajun, & Prakaypan, 2020; Sonprasarn, Chakartnarodom, Kongkajun, & Prakaypan, 2020). Previous work found that using polyurethane-based corn starch containing lithium perchlorate ($LiClO_4$) reduced the water absorption of fiber-reinforced cement composites (FRCC) due to the modified morphology of tobermorite, the crystalline calcium silicate hydrate (Chakartnarodom, Kongkajun, Chuankrerkkul, Ineure, & Prakaypan, 2019). Lagazzo, Vicini, Cattaneo, and Botter (2016) studied the use of fatty acid as a hydrophobic substance, these results showed enhanced water proofing of mortar. A study by Azarhomayun, Haji, Kioumarsi, and Shekarchi (2022) on aluminum powder and calcium stearate showed damp proofing of cement-based materials.

Accelerating additives, chemical admixtures introduced to cement mixtures, allow cement paste to set faster by increasing the rate of hydration and promoting early strength development. In FRCC processing, sufficient early strength is needed to enable earlier removal of formwork thus reducing the cycle time. The study of aluminum sulfate as an alkali-free accelerator for cement, Chen and Sun (2018), showed this shortened setting time, enhancing the degree of hydration and strength of cement paste due to the formation of a tight mesh structure in the ettringite phase.

Up to now, the generation of industrial waste has significantly increased worldwide. Due to environmental concerns, the recycling of industrial wastes as cement additives and/or as the raw material to reduce the usage of non-renewable raw materials has been extensively studied. Utilization of brick waste and soft sludge from the factory of FRCC in soil-cement brick production was studied by Kongkajun et al. (2020). The results showed improved strength and a reduction of the density and thermal conductivity of soil-cement bricks, in addition to the reduction of the usage of natural raw materials such as laterite. According to Sonprasarn, Chakartnarodom, Ineure, and Prakaypan (2019), Sonprasarn et al. (2020), Pahuswanno Chakartnarodom, Ineure, and Prakaypan (2019), and Pahuswanno et al. (2020), pretreated coal-fire bottom ash and FRCC waste could be used to enhance the hydration rate of Portland cement, the mechanical properties such as flexural strength and toughness of the FRCC product, and also reduce the natural raw materials used in FRCC production. Replacing Portland cement with paper sludge ash (PSA) by 12% reduced water absorption by 84% without a change in strength of materials (Wong, Barakat, Alhilali, Saleh, & Cheeseman, 2015). Pretreated crumb rubber was used as an additive to develop a hydrophobic rubberized cement paste (Chen, Shen, & Lee, 2021). The compressive and flexural strength of the concrete were maximized when 8% of short textile waste fibers containing cotton and polyester were added (Sadrolodabaee, Claramunt, Ardanuy & de la Fuente, 2021). Previous work by Li, Zhou, Zeng, Liu and Zhang (2019) added modified fenton paper sludge ash, which increased the bending and compressive strength after curing for 28 days.

At present, various kinds of industrial waste such as alumina waste and flue gas desulfurized gypsum (FGD) gypsum are blended during concrete production. Aluminum dross, a by-product of the aluminum melting process, consists mainly of alumina. Previous work by Mailar et al. (2016) showed that the utilization of recycled aluminum dross in concrete production yielded superior mechanical and durability properties. The FGD gypsum is a by-product generated from flue gas desulfurization process in coal-fired power plants. Yao, Wang, Liu, Yao and Wu (2019) investigated the utilization of fly ash, FGD gypsum, and carbide slag in a cementitious material to create a lightweight porous concrete. The results revealed that the synergistic effects of the various wastes improved the mechanical properties.

Waste recycling in cement and concrete products is an approach supporting circular and green economies. Thus, this work studied the effect of hybrid-aluminum additive (HAA) synthesized by both industrial wastes, aluminum dross and FGD gypsum, on the kinetics of the hydration reaction in FRCC.

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2. Materials and Methods

The hydration reaction between water and the OPC components is an exothermic chemical reaction as shown below (Askeland, Fulay, & Wright, 2010).

$$3CaO \cdot Al_2O_3 + 6H_2O \rightarrow Ca_3Al_2(OH)_{12} + Heat \quad (1)$$

$$3CaO + SiO_2 + (x+1)H_2O \rightarrow Ca_2SiO_4 \cdot xH_2O + Ca(OH)_2 + Heat \quad (2)$$

In this work, heat evolution from the exothermic reaction of hydration was analyzed based on the ASTM C186 (1998) standard. Heat of hydration measurements are important for assessing the rate of cement hydration or the potential temperature rise in cementitious materials.

The dry mixtures consisting of OPC, cellulose fibers, polyvinyl alcohol (PVA) fibers and sand were prepared based on the compositions shown in Table 1. To form the slurry, a water to cement ratio (w/c ratio) of 0.3 was used. The control formula is signified by REF in Table 1, a common production formula of fiber-cement composites used in wallboard applications. The HAA was synthesized from aluminum dross and FGD gypsum in a H_2SO_4 solution with Al^{3+}:SO_4^{2-} in a ratio of 1:1.5. The HAA was initiated and developed under a technology licensing agreement with Shera Public Company limited. The HAA was added to the dry mixture based on the amount of OPC in the mixture. The amounts of HAA added varied from 0 to 5 wt.% of total OPC weight in the dry mixture.

Instrumentation for studying the heat evolution from hydration reaction of the Portland cement and water composed of an insulated chamber, thermocouple, and temperature data logger, shown in Figure 2. The cement paste was kept in an insulated chamber. The effect of the HAA additive on the hydration reaction of OPC and water is determined by observing the temperature change within the containers over time. The temperature-data logger collected the temperature measured by the thermocouple and was recorded every 30 sec, for a total measurement time of 16 h.

Table 1. Formulations for the dry mixtures.

Raw Material	Formula Composition, wt%.					
	REF	**HAA1**	**HAA2**	**HAA3**	**HAA4**	**HAA5**
OPC type I	70	70	70	70	70	70
Sand	25	25	25	25	25	25
Cellulose fibers	4	4	4	4	4	4
PVA fibers	1	1	1	1	1	1
HAA (% of OPC weight added to the mixture)	0	1	2	3	4	5

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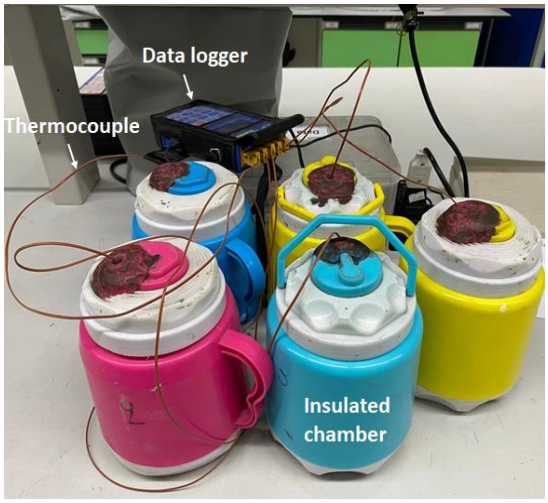

Figure 2. Schematic drawing of the instrument used for the heat of hydration test (Pahuswanno, et al., 2019; Sonprasarn et al., 2019).

3. Results and Discussion

The temperature profiles from the heat evolution of the hydration reactions are plotted in Figure 3. The temperatures initially increased with time, the cement paste was hydrated when reaching a maximum temperature (T_{max}) at the setting time (t_{max}), after which the temperature decreased significantly. When the amount of the HAA was increased from 1 to 5 wt.% of the OPC in the dry mixture, the temperature profile for all formulas using the HAA systematically shifted to shorter setting times (t_{max}) with higher maximum temperatures (T_{max}) compared to the control REF formula.

The average rate (R) of temperature change can be calculated from the difference of the maximum temperature (T_{max}) and the initial temperature (T_o), divided by the time to reach maximum temperature (t_{max}), as shown in the equation below (Sonprasarn et al., 2019).

$$R = \frac{T_{max} - T_o}{t_{max}} \quad (3)$$

The data T_{max}, $T_{initial}$, and t_{max} from Figure 3 are listed in Table 2 for each formula. The value R_i is the average rate of the temperature change for the formulas HAA1, HAA2, HAA3, HAA4 and HAA5, while R_{REF} is the average rate of the temperature change for the control REF formula. The average rate of the temperature change R_{REF}, R_1, R_2, R_3, R_4 and R_5 are calculated by Equation (3). The R_i/R_{REF} ratio represents the degree of the hydration reaction, with a value greater than 1 an enhancement.

The R_i/R_{REF} ratios for all were greater than unity with the utilization of the HAA, Table 2. Moreover, the R_i/R_{REF} ratios increased when the amount of the HAA increased. Plotting these ratios for each HAA formula, Figure 4, showed increasing the HAA content linearly improved the kinetics of the hydration reaction of the mixture due to the positive slope with a R^2 value of 0.9933. Thus, the HAA acted as an accelerating additive in the cement mixtures by shortening the setting time and enhancing the degree of the hydration reaction. This result corresponded to previous work by Chen and Sun (2018), that

showed that aluminum sulfate could shorten setting time and increase the early strength due to the formation of a tight mesh structure in the ettringite phase. The Al^{3+} and SO_4^{2-} ions in the HAA additive could react with tricalcium aluminate ($3CaO \cdot Al_2O_3$, C_3A) and gypsum phase ($CaSO_4 \cdot 2H_2O$) to produce a large number of ettringite ($Ca_3Al_2[SO_4]_3 \cdot [OH]_{12} \cdot 25H_2O$) crystals which promotes a shortened setting time for cement pastes.

However, when adding amounts of the HAA greater than 3%, the setting time was too rapid to shape the green samples and the maximum temperatures were very high (above 70°C) which could have a negative impact on the finished FRCC

Table 2. Data based on the temperature profiles in Figure 3, $T_{initial}$, T_{max}, t_{max}, R, and R_i/R_{REF}.

	REF	**HAA1**	**HAA2**	**HAA3**	**HAA4**	**HAA5**
T_o (°C)	28.6	27.7	28.6	30.9	34.4	33.9
T_{max} (°C)	66	69	69.2	69.2	72.1	75.7
Setting time, t_{max} (h)	6.7	6	5.21	4.57	4.15	4.05
$R = \frac{T_{max}-T_o}{t_{max}}$, (°C /h)	R_{REF} =5.58	R_1 =6.88	R_2 =7.79	R_3 =8.38	R_4 =9.08	R_5 =10.32
$\frac{R_i}{R_{REF}}$	1	1.15	1.31	1.41	1.53	1.73

product. It is well known that the rate and amount of heat generation due to hydration are important. Non-uniform thermal expansion/contraction due to a very large amount of heat can create undesirable stresses in cement. This may cause detrimental cracking, reducing its strength. Thus, the amount of 3% for the HAA is selected for further study.

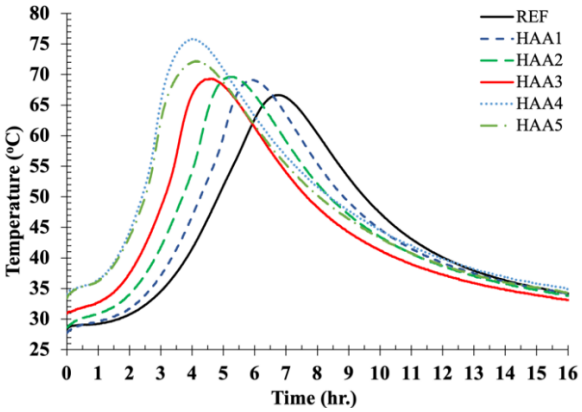

Figure 3. Temperature profile of the heat of hydration reaction of the mixtures.

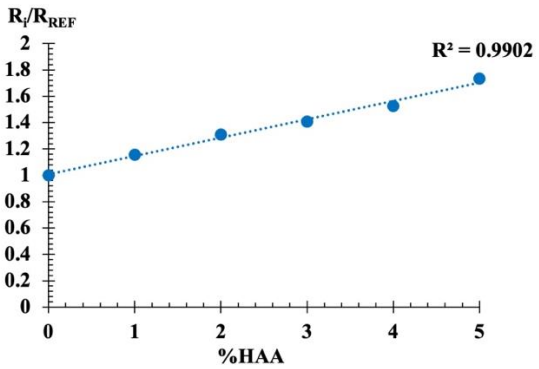

Figure 4. Effect of the % of HAA on the relative rate of temperature change R_i / R_{REF}.

4. Conclusions

This work studied the effect of the HAA synthesized by industrial wastes including aluminum dross and FGD gypsum on the kinetics of the hydration reaction of OPC in FRCC. It is found that the HAA systematically improved the hydration kinetics. However, the most suitable amount of HAA is 3% of the OPC weight in the dry mixture as higher amounts the setting time is too rapid with very high maximum temperatures. At this amount of the HAA the hydration reaction rate is significantly improved by 41%. This can dramatically alter setting times and thus cycle times reducing production times.

Acknowledgement

This work was supported by Shera Public Company Limited and the Faculty of Science and Technology, Thammasat University.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ORCID

Juthamat Nithipaiboon
https://orcid.org/0000-0003-3799-7273
Wichit Prakaypan
https://orcid.org/0000-0002-4904-1992
Parinya Chakartnarodom
https://orcid.org/0000-0002-4873-7285

Edward A Laitila
https://orcid.org/0000-0003-4868-9305
Nuntaporn Kongkajun
https://orcid.org/0000-0002-0856-5837

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