

# STI Focus

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National Institute of Science,  
Technology and Innovation

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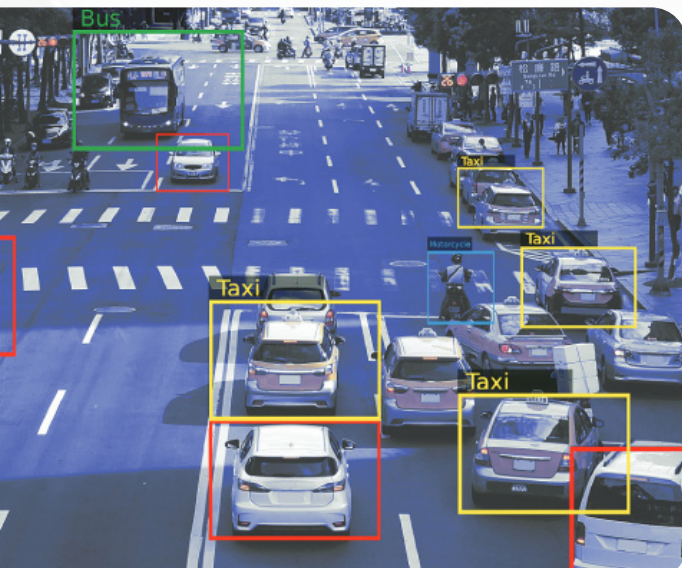


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# FOREWORD

Under the direction and long-term vision of Samdech Moha Borvor Thipadei HUN MANET, Prime Minister of the Kingdom of Cambodia, the Royal Government of Cambodia has implemented Phase I of the Pentagonal Strategy to carry out socio-economic development strategies. The Pentagonal Strategy consists of five key priorities, one of which is technology, and it is a key driver behind the mission of the Ministry of Industry, Science, Technology & Innovation (MISTI). To fulfill the national development goals and achieve Cambodia's vision for 2030 and 2050, MISTI has been mandated with the task to develop and improve Science, Technology, and Innovation (STI) throughout the country.

STI Focus is a bi-annual publication established by the National Institute of Science, Technology and Innovation (NISTI) under MISTI to further advance its mission and provide important research findings and insights to people of all ages. Through this STI Focus platform, scientists and researchers can share their scientific findings, technology trends, science policy, STEM education and careers, etc., with the public, which can be adopted to improve the productivity of micro, small, and mid-size businesses (MSMEs). From research findings to application, the team of expert writers holding master's and PhD degrees, have delved into the depths of this subject to bring an insightful and engaging collection of articles.

I would like to express my sincere appreciation to all the authors of this issue for your important contribution to the area of STI. I hope readers make good use of this information. The editorial team of STI Focus welcomes your contributions and looks forward to fruitful research and sharing in the next volume. I would like to take this opportunity to extend my sincere thanks to the editorial board, editor team, reviewers, and NISTI officers for making this issue possible.

Phnom Penh, 02 January 2024

**Minister**



**HEM Vandy**

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# EDITORIAL NOTE

The biannual publication, STI Focus, aims to promote the development of STI and enhance STI knowledge for the public. In its latest 2023 edition, this issue consists of two main sections: scientific findings and technology trends. The “Scientific Findings” section contains six articles addressing a variety of topics, including the characteristics of cassava flour quality, the utilization of eggshells as an adsorbent, the potential application of zirconium chalcogenide, the chemical properties of ginger, lemongrass, and bean sprouts, the histamine content in fermented fish products, and the seismic behavior of nuclear reinforced concrete structures. The “Technology Trends” section covers the latest technology from researchers, including the application of fungi as a potential biofertilizer, the removal of iron and arsenic by water purification technology, the functionality of bacteria in dye-decoloring, and traffic management using intelligent transportation systems.

The published articles in STI Focus were evaluated for their quality via a peer review process. Each of the figures and tables in the articles provides insight into the research findings and visualizes the latest technology. Furthermore, the digital version of our published STI Focus is open access and can be retrieved from the website of the Ministry of Industry, Science, Technology & Innovation (MISTI) or through the QR Code below.



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# SCIENTIFIC FINDINGS

# 1

## Characteristics of Cassava Flour Quality from Different Varieties

Chem Mouylin, Ph.D., Bioresources Engineering, University of Tsukuba, Japan

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### Highlight

- Rayong 72, KU 50, and Houybong 60 provide a high amount of starch
- The three varieties show similar characteristics of flour quality and are aligned with the standard of cassava
- The cyanide contained in cassava was found higher amount compared to the standard



Figure 1.1. Cassava plantation in Kompong Thom Province (The sampling area)

### I. Introduction

Cambodia is the 4<sup>th</sup> largest cassava producer in the South East Asia region accounting for 13.75 million tons in 2018 (Newby *et al.*, 2020) leading to one of the largest staple food crops for Cambodian people. Cassava flour is tiny particles from milled dried cassava root slices by fermented or unfermented process (Shittu *et al.*, 2016). An unfermented process method was used in this study, unfermented cassava flour also known as high-quality cassava flour (HQCF) (Amoa-Awua *et al.*, 2005). Although Cambodia produces and consume most cassava product, there are limited scientific evidence was found to state about standard, chemical, and

physical properties of both fresh and processed cassava flour.

The component of cassava flour quality especially hydrogen cyanide content is the most important indicator as mentioned in the quality factor of codex standard (FAO/WHO, 1995). Processing method, age at harvest, and cultivar condition affect the flour quality (Apea-Bah *et al.*, 2011; Bandna Address & Chand, 2012; Njankouo Ndam *et al.*, 2019). This study showed the current data on cassava flour quality from the 3 varieties based on selected regions, farm management, and particular processing methods.

## II. What is Cassava?

Cassava is a starchy root vegetable that grows underground from the cassava shrub. The shrub is scientifically known as *Manihot esculenta*, but it is more commonly called cassava. Cassava is a versatile crop that can be cooked, or processed into a variety of products, such as tapioca, flour, and chips. This study was conducted to determine the cassava flour quality which was processed from 3 different varieties of cassava, sources from Kampong Thom province known as Rayong 72, KU 50, and Houybong 60. As a result, all three varieties showed relatively similar results in moisture, ash, and titratable acidity except for starch content. Rayong 72 accounts for starch for  $81.70 \pm 0.26\%$ . Another result related to hydrogen cyanide, the toxic substance presented in cassava root and remained during the process, resulted in a remarkable amount ranging from 40mg/kg to 43mg/kg for cassava flour, higher than the standard provided by WHO 10mg/kg (CAC, 1995ab).

## III. Material and Methods



Figure 1.2. Cassava plantation

The three varieties of cassava are located in Sontok district, Kampong Thom province, and were growing in the same location.

### 3.1 Flour Processing

Cassava roots were peeled and soaked in water for 30mn, then sliced into 2-3mm thicknesses and placed



accordingly on the drier tray. The slice was dry at  $60 \pm 5^\circ\text{C}$  for 18h. All dried cassava chips were weighed accordingly and milled by a mini-milling machine then using 80mesh sieving.



Figure 2. Cassava root full of starchy content

### 3.2 Physicochemical Properties Determination

Moisture analysis was done by the official method AOAC 935.29, 1935, the sample was oven-dried at  $100 \pm 5^\circ\text{C}$  for 3h. Ash content determined by the method described in AOAC 942.05, 1942, 2g of sample heat at  $600^\circ\text{C}$  for 2h. Titratable acidity analysis was performed according to AOAC 935.57, 1935, calculated as acetic acid using phenolphthalein as pH indicator and titrated with 0.1M NaOH. Official method AOAC 920.40, 1920, was used to determine starch content. The picrate method was used for hydrogen cyanide which was described by (Bradbury *et al.*, 1999) and re-described by (Appenteng *et al.*, 2021), prepare picrate paper

*Table 1. Standard for cassava flour.*

No	Parameter	Standard
1	Moisture (max)	13%
2	Ash (max)	3%
3	Titrateable acidity (max)	1%
4	Starch (max)	65-70%
5	Hydrogen cyanide (max)	10 mg/kg

*Source: (Shittu et al., 2016)*

by dipping 3MM filter paper in a picrate solution which is a combination between 1.4% w/v picric acid and 2.5% w/v  $\text{Na}_2\text{CO}_3$  and air dry the paper then cut into 3cm x 1cm strips and stored in  $40^\circ\text{C}$  until use. Weighted 1g of sample put on filter paper with additional of 50 of Phosphate buffer pH 8 and 60 of linamarase or beta-glucosidase  $> 3\text{U/ml}$  then added 0.5ml of distilled water. Filter paper with sample was prepared into a transparent plastic bottle and picrate paper was immediately stick to the bottle neck and tight the bottle cap and left overnight (16 – 24h) at temperature  $30\text{-}40^\circ\text{C}$ . The picrate paper was soak into 5ml water in cuvette for 30mn and analyzed at a wavelength of 510nm and the result multiplied by 396.

#### IV. Result and Discussion

All three varieties showed relatively similar results in moisture, ash, and titratable acidity are below the maximum level of cassava flour standard except for starch content and hydrogen cyanide which is higher than the standard as shown in Table 2. Houybong 60 has the lowest acidity  $0.57 \pm 0.04\%$  and the higher starch content is Rayong 72 accounting for  $81.70 \pm 0.26\%$ , while hydrogen cyanide content is relatively high in all three varieties  $> 40\text{mg/kg}$  of the dry basis of cassava flour higher than WHO standard  $10\text{mg/kg}$  (FAO/

Table 2. The physicochemical properties of cassava flour in 3 different varieties.

Variety	Physicochemical				
	Moisture%	Ash%	Titratable acidity%	Starch%	Hydrogen cyanide (mg/kg)
Rayong 72	$4.84 \pm 0.14$	$1.22 \pm 0.18$	$0.83 \pm 0.03$	$81.70 \pm 0.26$	$42.00 \pm 1.00$
KU 50	$4.68 \pm 0.08$	$1.32 \pm 0.13$	$0.99 \pm 0.08$	$80.30 \pm 0.20$	$40.33 \pm 0.58$
Houybong 60	$4.55 \pm 0.07$	$1.21 \pm 0.23$	$0.57 \pm 0.04$	$81.33 \pm 0.25$	40.33 0.58

WHO, 1995) but still considerable for Indonesian cyanide content standard max  $40\text{mg/kg}$  (Djazuli *et al.*, 1999). Cassava is potentially toxic to eat raw or unprocessed. Therefore, it is important to process cassava tubers before consuming them. This can be done by drying them for at least a week, fermenting them, boiling them, or soaking them overnight. These processing methods help to minimize the toxicity of cassava (Westby, 2001).

#### V. Conclusion

All three varieties showed similar characteristics of flour quality and aligned with the standard of cassava flour given in Table 1. Except for the hydrogen cyanide which goes too high above the international standard, it will lead to health effects including nausea, vomiting, diarrhea, dizziness, and weakness (Akintonwa *et al.*, 1992). In the long-term consumption of high-level HCN can cause konzo and tropical ataxic neuropathy which are the problem with neurological conditions (Nzwalo *et al.*, 2011). HCN persists to remain even after processing so to ensure the limit of HCN in final products, proper processing technique must be applied. Furthermore, to encourage food safety and production of cassava flour in the region Cambodia should evaluate and establish its own HCN limited standard as in the case of Indonesia has adopted  $40\text{mg/kg}$  HCN for its standard.

## Reference:

- Akintonwa, A., & Tunwashe, O. L. (1992). Fatal cyanide poisoning from a cassava-based meal. *Human & experimental toxicology*, 11(1), 47–49. <https://doi.org/10.1177/096032719201100107>
- Amoa-Awua, W.K., Owusu, M. & Feglo, P. Utilization of Unfermented Cassava Flour for the Production of an Indigenous African Fermented Food, Agbelima. *World J Microbiol Biotechnol* 21, 1201–1207 (2005). <https://doi.org/10.1007/s11274-005-1441-7>
- Apea-Bah, F. B., Oduro, I., Ellis, W. O., & Safo-Kantanka, O. (2011). Factor Analysis and Age at Harvest Effect on the Quality of Flour from Four Cassava Varieties. In *World Journal of Dairy & Food Sciences* (Vol. 6, Issue 1).
- Bandna Address, C., & Chand, B. (2012). EFFECT OF PROCESSING ON THE CYANIDE CONTENT OF CASSAVA PRODUCTS IN FIJI. In *Journal of Microbiology* (Vol. 13, Issue 2).
- Djazuli, M., & Bradbury, J.H. (1999). Cyanogen content of cassava roots and flour in Indonesia. *Food Chemistry*, 65, 523-525.
- FAO/WHO (1995) Codex Standards for Edible Cassava Flour. In: Joint FAO/WHO Food Standards Programme, Codex Standards 176-1989, Codex Alimentarius Commission; Food and Agricultural Organization and World Health Organization of the United Nations, Rome
- Nzwalo, H., & Cliff, J. (2011). Konzo: from poverty, cassava, and cyanogen intake to toxico-nutritional neurological disease. *PLoS Neglected Tropical Diseases*, 5(6), e1051. <https://doi.org/10.1371/journal.pntd.0001051>
- Newby, J, Smith, D, Cramb, R, Delaquis, E & Yadav, L 2020, Cassava value chains and livelihoods in South-East Asia, a regional research symposium held at Pematang Siantar, North Sumatra, Indonesia, 1–5 July 2019, ACIAR Proceedings Series, No. 148, Australian Centre for International Agricultural Research, Canberra, 114 pp.
- Njankouo Ndam, Y., Mounjouenpou, P., Kansci, G., Kenfack, M. J., Fotso Meguia, M. P., Natacha Ngono Eyenga, N. S., Mikhaïl Akhobakoh, M., & Nyegue, A. (2019). Influence of cultivars and processing methods on the cyanide contents of cassava (*Manihot esculenta* Crantz) and its traditional food products. *Scientific African*, 5. <https://doi.org/10.1016/j.sciaf.2019.e00119>
- Shittu, Taofik & Alimi, Buliyaminu & Wahab, Bashira & Sanni, L. & Abass, Adebayo. (2016). Cassava Flour and Starch: Processing Technology and Utilization: Production, Processing and Technology. 10.1002/9781118992739.ch10a.
- Westby, A. (2001). Cassava utilization, storage and small-scale processing. In *Cassava: Biology, production and utilization* (pp. 281-300). Wallingford UK: Cabi.



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# 2

## Removal of Cadmium from River Water and Ground Water by Using Eggshell as Adsorbent

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### Highlight

- River water is the water on a surface that is formed from rain and other water resources
- Groundwater is the water that comes from the infiltration of water on the ground surface
- Cadmium (Cd) is a soft, silvery-white metal with the atomic number 48 in the 12<sup>th</sup> group of block-d and the 5<sup>th</sup> period of the periodic table
- Eggshell is the waste that comes from kitchens and other food production units and it has  $\text{CaCO}_3$  quantity, which can function as adsorbent



Using eggshell as adsorbent

### I. Introduction

Water, encompassing rivers, oceans, streams, and lakes, serves various purposes such as public supply, irrigation, and addressing water scarcity. Approximately 80% of river water is utilized for these purposes (Kimberly Mullen, 2023). However, as pollution levels increase, the demand for water also rises. To combat this issue, groundwater is tapped into as an alternative water resource (ODC, 2016). Despite its

seemingly clean appearance, groundwater can contain harmful elements like Arsenic, Cadmium, or Fe (heavy metals). These metals pose a significant obstacle to accessing safe water. Groundwater plays a crucial role in satisfying domestic, agricultural, and industrial water needs in certain countries. Groundwater and rivers become contaminated because of human and industrial activities, with heavy metals being the most significant pollutants in this regard. Cadmium, a naturally occurring substance in the earth's crust, is commonly used in paints, pigments, coatings, and inexpensive jewelry. Consequently, industrial plants contribute to its presence in water resources. Due to its tendency to accumulate in the kidneys, cadmium poses a risk of kidney disease, as it is not easily eliminated from the body. Adopting the shelling method can be regarded as an effective approach, as it involves utilizing waste and recycled materials to extract metal from water resources, leading to waste reduction and environmental preservation. This study's objectives were (i) to determine the cadmium concentration in river and ground water with different places. (ii) to study the cadmium adsorption capacity by using eggshell and (iii) to compare the cadmium concentration between river and ground water by following Ministry of Mines and Energy Cambodia's standard.

## II. Material and Method

### 2.1 Sample Collection and Preparation

The river water samples were collected from Cham village, Prek Thmey commune, Koh Thom district, Kandal province. Groundwater samples were collected from Toul Toteung 3 village, Toul Toteung commune, Prey Nob district, Sihanoukville province as shown in figure 1. Samples were collected on March 11, 2023 by following the method described by (Hamzat A, 2021).

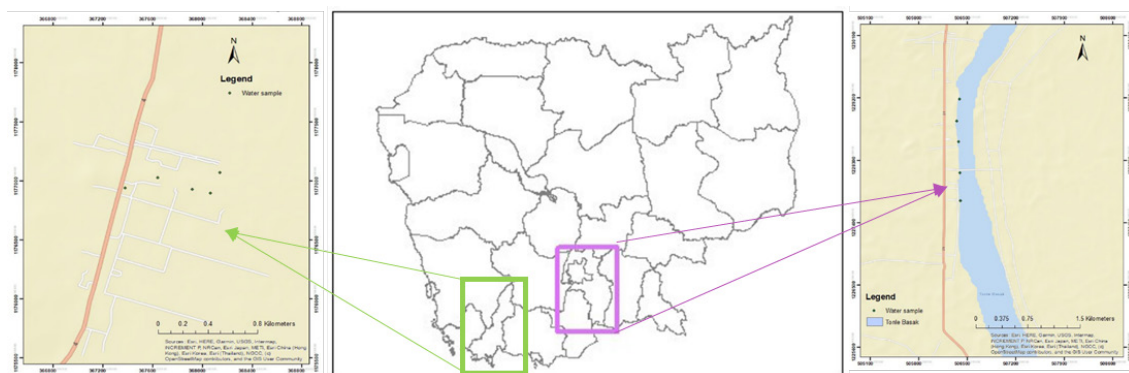


Figure 1. The location of river water and groundwater sample collection

The samples were filtered by using 110 mm filter paper and 0.20  $\mu\text{m}$  syringe filter paper. The filtrate samples were added 0.5 mL of 65% nitric acid and kept in the refrigerator (4°C) for future analysis.

Eggshell waste was collected from a grocery store in the Royal University of Phnom Penh. The eggshell was washed with tap water 3 times. After that, the eggshells were placed under sunlight until they dried and grind it to be a powder.

### 2.2 Adsorption Experiment

5g of eggshell was put in 50 mL of each water sample with the contact time of 90min. The mixture was shaken at 200 rpm using a magnetic hotplate stirrer and the solution was filtered through 110 mm and 0.20  $\mu\text{m}$  syringe filter paper. The solutions were kept for calcium analysis by using Flame Atomic Adsorption

### 2.3 Statistics Analysis

The experiments were done in triplicate and the results were indicated as mean and standard deviation with confidence interval 95% (Mean  $\pm$  SD). Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) and Microsoft Excel was used to calculate the result and plot the graph.

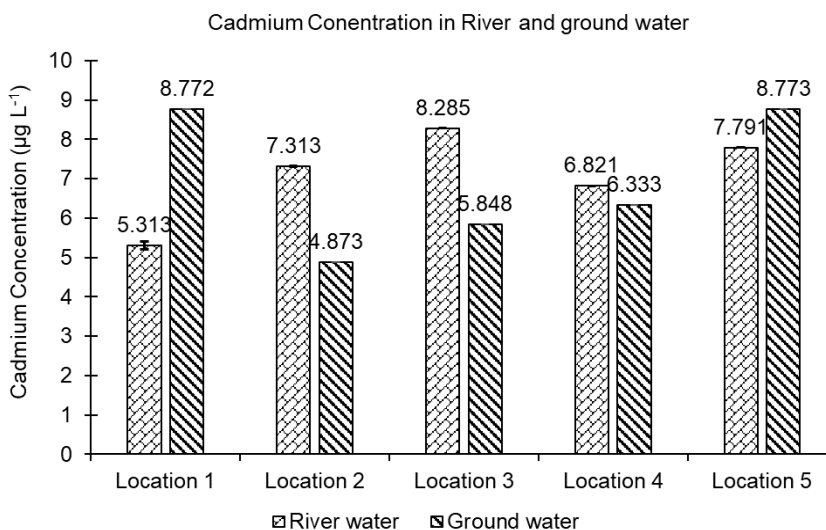


Figure 2. The cadmium concentration in river and ground water

## III. Result and Discussion

### 3.1 The Cadmium Concentration in River and Ground Water

In figure 2 showed that, the cadmium concentration was ranging from  $5.313 \pm 1.02 \mu\text{g L}^{-1}$  to  $8.285 \pm 0.005 \mu\text{g L}^{-1}$  and the highest concentration in location 3 of the river water sample with the value of  $8.285 \pm 0.005 \mu\text{g L}^{-1}$  in Cham village of Kandal province. In addition, the cadmium concentration in ground water samples were ranging from  $4.873 \pm 0.002 \mu\text{g L}^{-1}$  to  $8.773 \pm 0.002 \mu\text{g L}^{-1}$  and the highest at location 1 and 5 with both value of  $8.773 \pm 0.002 \mu\text{g L}^{-1}$  in Toul Toteung 3 village of Sihanoukville province. These results indicated that the cadmium concentration in both provinces was higher than the concentration of Cadmium metal in the drinking water standard of the Ministry of Industry and Mines. In both water resources can be polluted due to the discharge of various wastes from industries, the usage of chemical fertilizers and pesticide in agriculture and wastes disposal near the water resource (Tzanakakis *et al.*, 2020). Hence, there is a potential concern regarding the quality of river water in Kandal province and ground water in Sihanoukville province, which may pose a risk when used for consumption. Therefore, it is crucial to find effective methods to purify the water before consuming it.

### 3.2 The Cadmium Concentration After Adsorbed by Using Eggshell

The utilization of a 5 g dose of eggshell and a contact time of 90 minutes has proven to be highly effective in adsorbing cadmium. Figure 3 illustrates the cadmium adsorption. The percentage of cadmium concentration removal in river water at each location ranged from 75.018% to 90.848%, while groundwater in Sihanoukville province exhibited removal rates between 72.212% and 89.989%. The varying removal percentages can

be attributed to distinct sources of water pollutants and the presence of other metal ions in the samples, which may hinder the adsorption capacity of the eggshell agent (Tzanakakis *et al.*, 2020). In addition, eggshell possesses carbonyl, carboxylic, and hydroxyl functional groups that have a significant impact on the adsorption of metals (Abatan, O. G., *et al.*, 2020). The porous structure of eggshell provides a large surface area for adsorption, is mainly composed of calcium carbonate, and presents a neutral surface charge at pH 7 (de Oliveira Zonato, R., 2022).

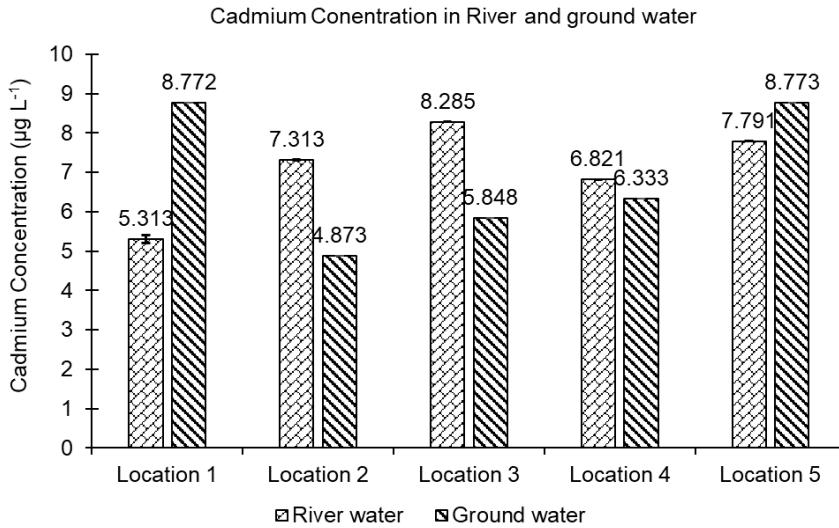


Figure 3. The cadmium concentration after adsorbed by using eggshell

### 3.3 The Comparison of Cadmium Removal with Drinking Water Standard of the Ministry of Industry, Mines and Energy

Table 1 showed the comparison of Cadmium removal with drinking water standard. This comparison shows that there are no significant differences between all locations in river water and ground water ( $p < 0.05$ , T-Test, One sample). However, if comparing in each location to the standard drinking water of the Ministry of Mines and Energy (2004), there are all significant differences by the concentration of cadmium in river water and ground water lower than the drinking water standard of the Ministry of Mines and Energy. According to this result, the eggshell is a very useful waste for absorbent material or it can be efficiently and effectively converted into valuable industrial products.

Location	Number of sample	Cd final conc in River water of Kandal province	Cd final conc in ground water of Kandal province	Drinking water standard
Location1	4	$0.486^b \pm 0.001 \mu\text{g L}^{-1}$	$1.948^b \pm 0.000 \mu\text{g L}^{-1}$	3 <sup>a</sup>
Location2	4	$1.462^b \pm 0.000 \mu\text{g L}^{-1}$	$0.488^b \pm 0.000 \mu\text{g L}^{-1}$	3 <sup>a</sup>
Location3	4	$1.948^b \pm 0.000 \mu\text{g L}^{-1}$	$0.975^b \pm 0.001 \mu\text{g L}^{-1}$	3 <sup>a</sup>
Location4	4	$0.974^b \pm 0.000 \mu\text{g L}^{-1}$	$0.975^b \pm 0.001 \mu\text{g L}^{-1}$	3 <sup>a</sup>
Location5	4	$0.974^b \pm 0.000 \mu\text{g L}^{-1}$	$2.437^b \pm 0.000 \mu\text{g L}^{-1}$	3 <sup>a</sup>

Table 1. The comparison of Cadmium removal with drinking water standard

## IV. Conclusion

The water samples in both provinces were contaminated with cadmium metal with a high concentration of  $8.285 \pm 0.005 \mu\text{m L}^{-1}$  in river water and  $8.773 \pm 0.002 \mu\text{g L}^{-1}$  in ground water. The high percentage of

cadmium removal was 90.848% in river water and 89.989% in ground water. In addition, the remaining cadmium concentration value is below the standard drinking water of 3 µg L<sup>-1</sup>, by the Ministry of Mines and Energy. According to this result, the application of eggshell as an adsorbent for removing heavy metals from river water, ground water, and contaminated samples has significant potential. Instead of discarding eggshells as waste, they can be efficiently and effectively converted into valuable industrial products.

### Reference:

- Abatan, O. G., Alaba, P. A., Oni, B. A., Akpojevwe, K., Efeovbokhan, V., & Abnisa, F. (2020). Performance of eggshells powder as an adsorbent for adsorption of hexavalent chromium and cadmium from wastewater. *SN Applied Sciences*, 2, 1-13.
- de Oliveira Zonato, R., Estevam, B. R., Perez, I. D., dos Santos Ribeiro, V. A., & Boina, R. F. (2022). Eggshell as an adsorbent for removing dyes and metallic ions in aqueous solutions. *Cleaner Chemical Engineering*, 2, 100023.
- Hamzat A, D.-K. A. and I. R. (2021). Determination of heavy metals associated with surface water of Bakajeba reservoir, Niger state, NigeNo Title. *International Journal of Fisheries and Aquatic Studies*. <https://www.fisheriesjournal.com/archives/2021/vol9issue6/PartD/9-5-58-583.pdf>
- Kimberly Mullen, C. (2023). Information on Earth's Water. NGWA. <https://www.ngwa.org/what-is-groundwater/About-groundwater/information-on-earths-water>
- MINISTRY OF INDUSTRY MINES AND ENERGY. (2004). DRINKING WATER QUALITY STANDARDS. MINISTRY OF INDUSTRY MINES AND ENERGY. <https://rdic.org/wp-content/uploads/2014/12/MIME-Drinking-Water-Quality-Standards-2004-en.pdf>
- Ministry of Rural Development. (2019). National Action Plan Rural Water Supply, Sanitation and HygieneNo Title. Ministry of Rural Development. [https://www.unicef.org/cambodia/media/2741/file/NAP\\_II.pdf\\_.pdf](https://www.unicef.org/cambodia/media/2741/file/NAP_II.pdf_.pdf)
- ODC. (2016). Ground waterNo Title. 2016. <https://opendevelopmentcambodia.net/topics/groundwater/>
- Tzanakakis, V. A., Paranychianakis, N. V., & Angelakis, A. N. (2020). Water Supply and Water Scarcity. In *Water* (Vol. 12, Issue 9). <https://doi.org/10.3390/w12092347>



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# 3

## Zirconium Chalcogenide Materials: Potentials for Technological Applications Despite Degradation in Ambient Air

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### Highlight

- Similar to other promising ultrathin 2D materials such as black phosphorus, tin dichalcogenides (SnS<sub>2</sub>, SnSe<sub>2</sub>), Zirconium chalcogenides are observed to degrade in ambient air as observed by using optical microscope and atomic force microscope
- Despite degradation, these 2D materials have been shown to provide exceptional properties that are potential in technological applications.

### I. Introduction

Interest in two-dimensional (2D) materials such as hexagonal boron nitride (hBN), black phosphorus (BP) and transition-metal dichalcogenides (TMDs) since the discovery of graphene (Novoselov, *et al.*, 2004) by two Nobel laureates (Andre Geim and Konstantin Novoselov; Nobel prize in Physics in 2010) has significantly increased due to their unique structures and properties. The potential uses of 2D materials in industries range from optoelectronics, sensors, spintronics, superconductors and energy storage to thermoelectric and topological insulator devices. Like other 2D materials, zirconium chalcogenides such as ZrS<sub>3</sub>, ZrSe<sub>3</sub>, ZrS<sub>2</sub>, ZrSe<sub>2</sub> consist of layers that attract each other by weak van-der-Waals force.

This results in easy separation of each layer down to less than one nano-meter thick. The process can be done by using mechanical exfoliation, where simple scotch tape is used to make mono- or few-layer 2D samples from its bulk counterpart onto a substrate such as SiO<sub>2</sub>/Si or polymer polydimethylsiloxane (PDMS). Atomically thin zirconium chalcogenides are widely known to exhibit more interesting properties as compared to their bulk counterparts.

However, they are observed to degrade in ambient air. In this article, we provide scientific evidence of degradation of thin-layer zirconium chalcogenides by using optical microscope (OM) and atomic force microscope (AFM), which are available at the Optoelectronics and Advanced Materials Lab, Royal University of Phnom Penh, aside from several exceptional properties discovered by other scientists.

### II. Observation of Degradation in 2D Zirconium Chalcogenides

Zirconium-based materials such as ZrS<sub>3</sub>, ZrSe<sub>3</sub>, ZrS<sub>2</sub> and ZrSe<sub>2</sub> are widely known for their promising properties

appropriate for cathode material for Li primary thermal batteries (Giagloglou, *et al.*, 2016), efficient catalyst (Xie, *et al.*, 2014), thermoelectricity (Ding, *et al.*, 2017; Qin, 2017), potential for photoemission devices (Pacilé, 2007), photodetectors (Xiong, 2015) and high performance Schottky solar cells (Li, *et al.*, 2011). However, unlike graphene and other TMDs such as MoS<sub>2</sub> and WS<sub>2</sub> that are stable in air, they significantly degrade. In other words, the materials are unstable. There exist several techniques for investigating the stability of semiconducting materials such as scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), Raman spectroscopy, photoluminescence (PL) spectroscopy, OM and AFM (Edmonds *et al.*, 2015; Favron, *et al.*, 2015; Gao, *et al.*, 2016). In the current study, atomically thin samples were made onto SiO<sub>2</sub>/Si substrates with 280 nm-thick oxide layer (Sriv, Kim & Cheong, 2018) from ZrS<sub>3</sub>, ZrSe<sub>3</sub>, ZrS<sub>2</sub> and ZrSe<sub>2</sub> single crystals by using mechanical exfoliation (Novoselov, *et al.*, 2004). OM and AFM (Nanosurf C3000) were used to investigate whether thin samples are stable. According to Figure 1, optical images of mechanically exfoliated thin ZrS<sub>2</sub>, ZrSe<sub>2</sub>, ZrS<sub>3</sub>, and ZrSe<sub>3</sub> indicate significant degradation after being left in air. Specifically, optical image of ZrS<sub>2</sub> shows significant degradation after 8 days (Figure 1.b) being left in air as compared to that of (a). Similarity is observed in ZrSe<sub>2</sub> (Figure 1.c-d) for even 3 hours, while in the case of ZrS<sub>3</sub> in Figure 1.e and f, one part of the sample completely disappeared after 21 days. In Figure 1.g and h, the optical images of ZrSe<sub>3</sub> significantly differ from each other due to degradation. Similar evidence is clearly confirmed in the AFM results of Figure 2. Fast degradation results of zirconium chalcogenides suggest vacuum consideration for future characterizations and appropriate techniques for device fabrications by using these materials.

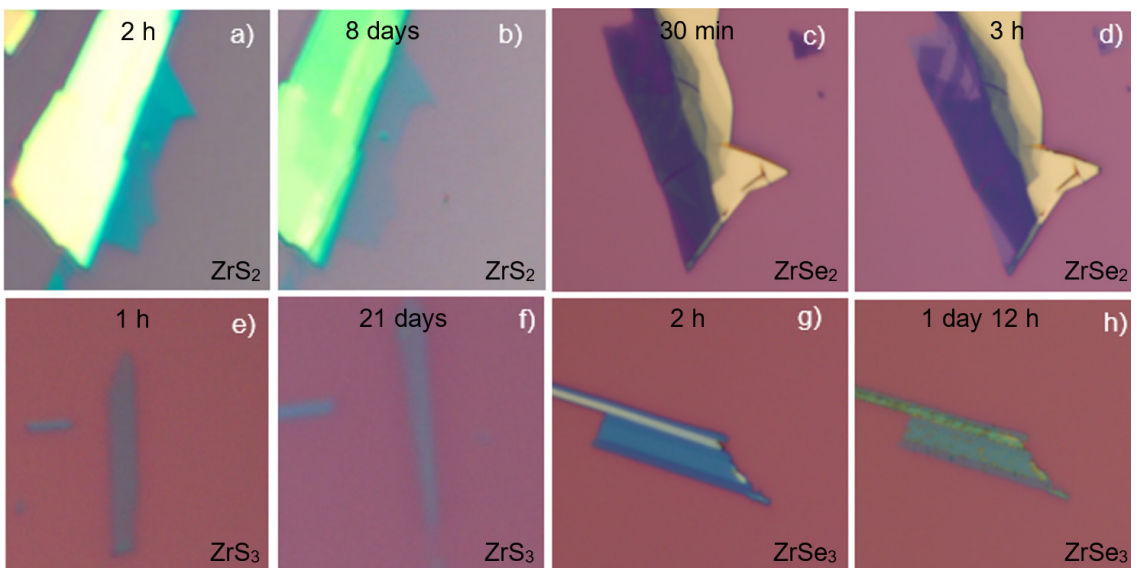


Figure 1. Optical images of ZrS<sub>2</sub>, ZrSe<sub>2</sub>, ZrS<sub>3</sub>, and ZrSe<sub>3</sub> (a-h)

### III. Technological Applications Based on Zirconium Chalcogenides

2D material such as graphene and TMDs have attracted research interests due to exceptional quantum effects and fascinating properties, which have led to several industrial applications (Tiwari, *et al.*, 2020; Dhinakaran, *et al.*, 2020; Mueller, *et al.*, 2018). Being transition metal chalcogenides of similar class, Zirconium based materials such as ZrS<sub>2</sub>, ZrSe<sub>2</sub>, ZrS<sub>3</sub>, and ZrSe<sub>3</sub> were shown to have exceptional properties for technological applications. The following figure shows several of them that are of great interest of scientific community and semiconductor companies

#### IV. Conclusion

In summary, the degradation of zirconium chalcogenides was observed by using an OM and AFM. Among the studied zirconium-based materials, atomically thin  $\text{ZrS}_2$ ,  $\text{ZrSe}_2$ ,  $\text{ZrSe}_3$  samples are shortly degraded in ambient air, whereas  $\text{ZrS}_3$  is relatively more stable. This suggests that device fabrications and future characterizations of these materials require consideration of heterostructures to cover them and vacuum conditions, respectively, despite their potentials for technological applications.

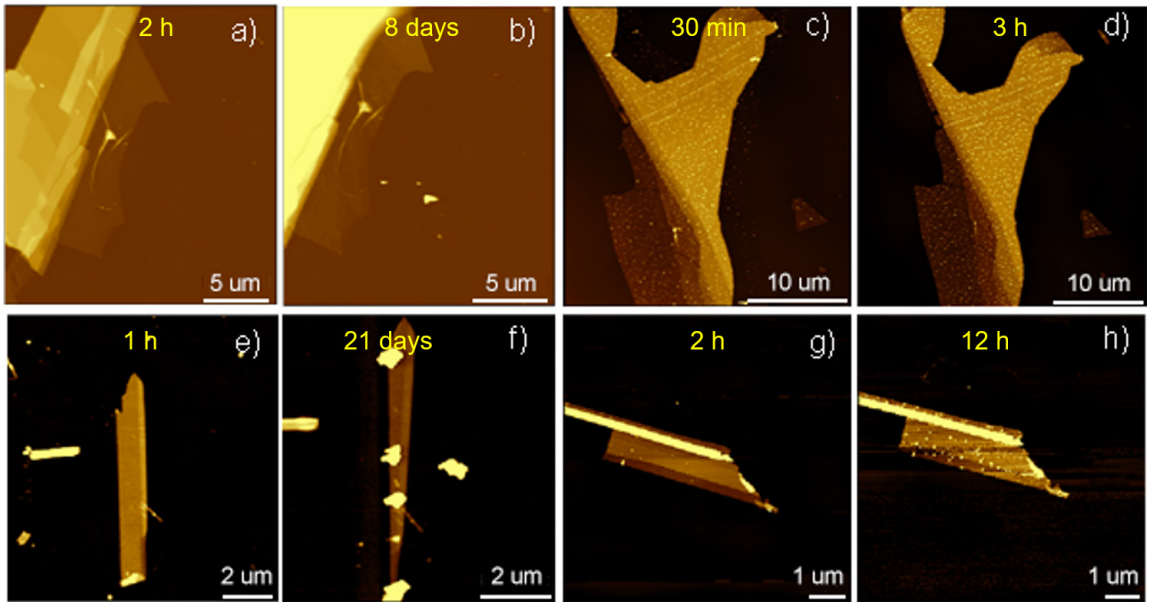


Figure 2. AFM images of  $\text{ZrS}_2$ ,  $\text{ZrSe}_2$ ,  $\text{ZrS}_3$ , and  $\text{ZrSe}_3$  (a-h)

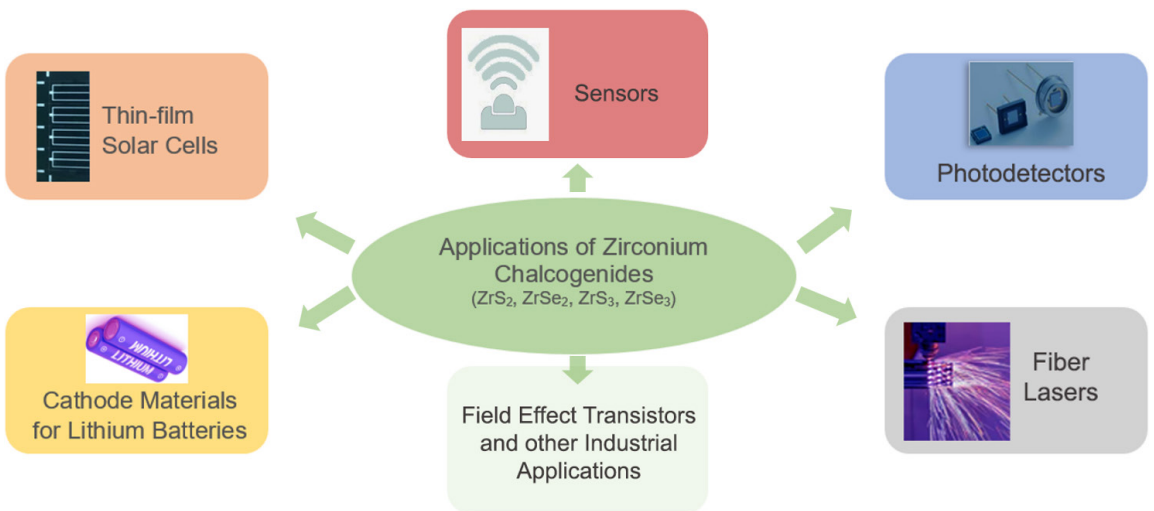


Figure 3. Applications of Zirconium Chalcogenides (Giagloglou, *et al.*, 2016; Xiong, 2015; Li, *et al.*, 2011; Li, Lv, *et al.*, 2019; Katarzyna, *et al.*, 2023).

## Reference:

- Dhinakaran, V., Lavanya, M., Vigneswari, K., Ravichandran, M., & Vijayakumar, M. D. (2020). Review on exploration of graphene in diverse applications and its future horizon. *Materials Today*: <https://doi.org/10.1016/j.matpr.2019.12.369>
- Ding, G., Chen, J., Yao, K., & Gao, G. (2017). Convergence of separate orbits for enhanced thermoelectric performance of layered ZrS<sub>2</sub>. *New Journal of Physics*, 19(7), 073036. <https://doi.org/10.1088/1367-2630/aa7b58>
- Edmonds, M. T., Tadich, A., Carvalho, A., Ziletti, A., O'Donnell, K. M., Koenig, S. P., Coker, D. F., Özyilmaz, B., Neto, A. H. C., & Fuhrer, M. S. (2015). Creating a stable oxide at the surface of black phosphorus. *ACS Applied Materials and Interfaces*, 7(27), 14557–14562. <https://doi.org/10.1021/acsami.5b01297>
- Favron, A., Gauffrès, E., Fossard, F., Heureux, A. P., Tang, N. Y., Lévesque, P. L., Loiseau, A., Leonelli, R., Francoeur, S., & Martel, R. (2015). Photooxidation and quantum confinement effects in exfoliated black phosphorus. *Nature Materials*. 14, 826–832. <https://doi.org/10.1038/nmat4299>
- Gao, J., Li, B., Tan, J., Chow, P., Lu, T. M., & Koratkar, N. (2016). Aging of Transition Metal Dichalcogenide Monolayers. *ACS Nano*, 10(2), 2628–2635. <https://doi.org/10.1021/acsnano.5b07677>
- Giagloglou, K., Payne, J. L., Crouch, C., Gover, R. K. B., Connor, P. A., & Irvine, J. T. S. (2016). Zirconium Trisulfide as a Promising Cathode Material for Li Primary Thermal Batteries. *Journal of The Electrochemical Society*, 163(14), A3126–A3130. <https://doi.org/10.1149/2.1351614jes>
- Island, J. O., *et al.* (2017). Electronics and optoelectronics of quasi-1D layered transition metal trichalcogenides. *2D Materials*, 4(2), 1–31. <https://doi.org/10.1088/2053-1583/aa6ca6>
- Island, J. O., *et al.* (2014). Effective Passivation of Exfoliated Black Phosphorus Transistors against Ambient Degradation. *Nano Letters*, 14(12), 6964–6970. <https://doi.org/10.1021/nl5032293>
- Katarzyna, D., Adil, R., Sergey, R., Michelle, W., Ludwig, B., Alexander, B., Janusz, S. & Grzegorz, C. (2023). Study of ZrS<sub>3</sub>-based field-effect transistors toward the understanding of the mechanisms of light-enhanced gas sensing by transition metal trichalcogenides. *Materialstoday Communications*. 34, <https://doi.org/10.1016/j.mtcomm.2023.105379>
- Kim, S., Kim, Y. J., & Ryu, W. H. (2021). Zirconium disulfides as an electrode material alternative for Li-ion batteries. *Applied Surface Science*, 547, 1–6. <https://doi.org/10.1016/j.apsusc.2021.149029>
- Li, L., Wang, H., Fang, X., Zhai, T., Bando, Y., & Golberg, D. (2011). High-performance Schottky solar cells using ZrS<sub>2</sub> nanobelt networks. *Energy & Environmental Science*, 4(7), 2586. <https://doi.org/10.1039/c1ee01286j>
- Li, L., Lv, R., Wang, J., Chen, Z., Wang, H., Liu, S., Ren, W., Liu, W. & Wang, Y. (2019). Optical Nonlinearity of ZrS<sub>2</sub> and Applications in Fiber Laser. *Nanomaterials*, 9(3), 315. <https://doi.org/10.3390/nano9030315>
- Mueller, T., Malic, E. Exciton physics and device application of two-dimensional transition metal dichalcogenide semiconductors. *npj 2D Mater Appl* 2, 29 (2018). <https://doi.org/10.1038/s41699-018-0074-2>
- Novoselov, K. S. *et al.* (2004). Electric field effect in atomically thin carbon films. *Science* 306, 666–669. <https://doi.org/10.1126/science.1102896>
- Pacilé, D., Papagno, M., Lavagnini, M., Berger, H., Degiorgi, L., & Grioni, M. (2007). Photoemission and optical studies of ZrSe<sub>3</sub>, HfSe<sub>3</sub>, and ZrS<sub>3</sub>. *Physical Review B*, 76(15). <https://doi.org/10.1103/physrevb.76.155406>
- Qin, D., Ge, X.-J., Ding, G., Gao, G., & Lü, J.-T. (2017). Strain-induced thermoelectric performance enhancement of monolayer ZrSe<sub>2</sub>. *RSC Adv.*, 7(75), 47243–47250. <https://doi.org/10.1039/c7ra08828k>
- Sriv, T., Kim, K. & Cheong, H. Low-Frequency Raman Spectroscopy of Few-Layer 2H-SnS<sub>2</sub>. *Sci Rep* 8, 10194 (2018). <https://doi.org/10.1038/s41598-018-28569-6>
- Tiwari, S. K., Sahoo, S., NannanWang, & Huczko, A. (2020). Graphene Research and their Outputs: Status and Prospect. *Journal of Science: Advanced Materials and Devices*. <https://doi.org/10.1016/j.jsamd.2020.01.006>
- Xie, J., Wang, R., Bao, J., Zhang, X., Zhang, H., Li, S., & Xie, Y. (2014). Zirconium trisulfide ultrathin nanosheets as effi-

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cient catalysts for water oxidation in both alkaline and neutral solutions. *Inorg. Chem. Front.*, 1(10), 751–756. <https://doi.org/10.1039/c4qi00127c>

Xiong, W.-W., Chen, J.-Q., Wu, X.-C., & Zhu, J.-J. (2015). Visible light detectors based on individual ZrSe<sub>3</sub> and HfSe<sub>3</sub> nanobelts. *Journal of Materials Chemistry C*, 3(9), 1929–1934. <https://doi.org/10.1039/c4tc02492c>



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# 4

## Determination of Sodium Dithionite in Ginger, Lemongrass, and Bean Sprout

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### Highlight

- The purpose of this research is to determine the concentration of sodium dithionite in ginger, lemongrass, and bean sprouts
- UV-Spectrophotometry is used to quantify sodium dithionite concentration
- Sodium Dithionite concentrations in three samples were higher than FSANZ (10mg/kg) and lemongrass had the highest amount of sodium dithionite.as adsorbent



Figure 1. (a) Ginger (*Zingiber officinale*), (b) Lemongrass (*Cymbopogon citratus*) and (c) Bean Sprout (*Vigna radiata*)

### I. Introduction

Vegetables are significant sources of nutrition due to their high nutrient content of vitamins, such as vitamins B, C, and K, and minerals such as calcium, potassium, and magnesium, as well as dietary fiber (Yahia *et al.*, 2018). Fresh vegetables can be damaged and rotten, caused of oxygen, moisture, light, heat, and microorganisms that can cause human foodborne diseases (Nunn, 2019). To prevent vegetables from rotten, people can store them at low temperatures, and add chemicals or substances known as food additives. Sodium dithionite or sodium hydrosulfite is being widely used in the food industry by minimizing its amount to keep vegetables, fruit, and food fresh with the food additive code E-222 to keep them from being damaged by bacteria (Mirza *et al.*, 2015 and Chen *et al.*, 2016). The maximum concentration of sodium hydroxide in vegetables is set

to not exceed the average value of 10 mg/kg (Te *et al.*, 2013). If used in quantities greater than 30g/Kg, it can have a significant impact on the consumers' health such as abdominal pain, nausea, dizziness, diarrhea, dermatitis, difficulty breathing, low blood pressure, and shock and tremors for people with severe food allergies or asthma. More importantly, people can be unconscious and potentially fatal if ingested more than safety standards (Sila *et al.*, 2017). Therefore, this study aims to determine the concentration of Sodium Dithionite in Ginger, Lemongrass, and Bean Sprouts from Doeurm Kor Market located in Phnom Penh, Cambodia by using UV spectrophotometry.

## II. Materials and Method

### 2.1 Chemicals

All the chemicals used were analytical grade. Naphthol Yellow S was prepared by weighing 0.8 g dissolving with distilled water and adding 10.0 ml of ammonia hydroxide solution in a 1000.0 ml volumetric flask and was kept in amber glass due to the light sensitivity of this reagent. 1.0 M NaOH was used as the solvent for Sodium dithionite standard solution preparation.

was diluted to 20.0, 40.0, 80.0, 120.0, 160.0, and 200.0 mg/kg using 1.0 M NaOH as solvent. 10.0 mL of Naphthol Yellow S was added to each diluted standard solution and its final volume was adjusted to 25.0 mL with distilled water. The absorbance was measured at 502 nm with UV (GENESYS 10S UV-VIS) to make a standard curve. Six sodium dithionite concentrations were measured in triplicate.

### 2.3 Samples

Three vegetables, namely Ginger (*Zingiber officinale*), Lemongrass (*Cymbopogon citratus*), and Bean Sprout (*Vigna radiata*), were collected from Doeurm Kor Market in Phnom Penh City, Cambodia. All samples were randomly selected and kept in the polyethylene bag and transported to the laboratory. Samples were analyzed as fresh without rinsing.

### 2.4 Sodium Dithionite Determination

30.0 g of each sample were weighed and 60.0 mL of distilled water was added. The mixture was then filtered through a no. 1 filter paper (Whatman, England). 10.0 ml of filtrate was transferred into a 25.0 mL volumetric flask and 10.0 ml of Naphthol Yellow S then the final volume of sample solution

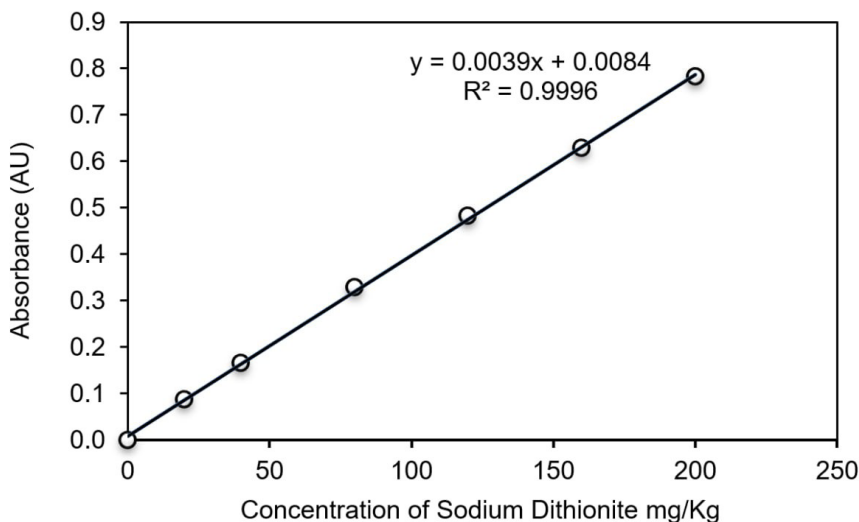


Figure 2. Standard Curve of Sodium Dithionite from 20.0 to 200.0 mg/kg measured at 502 nm

### 2.2 Standard Curve

Sodium dithionite stock solution (1000 mg/kg)

was adjusted to 25.0 mL with distilled water. The sodium dithionite concentration was determined

from absorbance measurement at 502 nm by UV (GENESYS 10S UV-VIS). All experiments were done in triplicate.

## 2.5 Accuracy

The accuracy was determined by the spiked method where a known amount of standard formaldehyde solution was added to samples to give concentration of 80.0 mg/kg and was analyzed by the proposed methods. An average percent recovery obtained from three experiments was calculated.

## III. Result and Discussion

### 3.1 Standard Curve

The relation between sodium dithionite concentration and absorbance was linear with  $R^2 = 0.9996$ , and an equation of the standard curve was  $y = 0.0039x + 0.0084$ , where  $y$  is in absorbance units (AU) and  $x$  is sodium dithionite concentration in mg/kg Figure 2. Limit of detection (LOD) of 1.55 mg/kg and limit of quantification (LOQ) of 5.15 mg/kg were achieved and calculated based on the European Commission Decision 2002/657/EC (2002) (European Commission, 2002). The average recovery percentage of Sodium Dithionite was obtained using the spiked method from three samples. Table 1. showed that the recovery percentages exceeded 95% which was between 80 - 120% and demonstrated the good accuracy of the method.

Table 1. Recovery percentage of Sodium Dithionite in samples using spiked method at 80.0 mg/kg

Sample	Added Standard Sodium Dithionite (mg/kg)	Unspiked (mg/kg)	Spiked (mg/kg)	Recovery Percentage (%)
Ginger	80.000	99.002	180.096	101.368
Lemongrass	80.000	98.487	173.820	94.166
Bean Sprout	80.000	46.320	124.562	97.803

Table 2. Sodium Dithionite concentration in samples (mg/Kg)

Sample	N	Mean ± SD (mg/kg)	CI 95%	T-test 95%
Ginger	3	100.961±1.033	100.961±2.566	152.513
Lemongrass	3	110.863±2.795	110.863±6.944	62.498
Bean Sprout	3	37.529±3.392	37.529±8.426	14.057

### 3.2 Determination of Sodium Dithionite Ginger, Lemongrass, and Bean Sprout

The mean sodium dithionite in fresh samples of three vegetables, ginger, lemongrass, and bean sprout, are summarized in Table 2. The sodium dithionite concentrations ranked the vegetables in the order lemongrass (110.863 mg/Kg) > ginger (100.961 mg/kg) > bean sprout (37.529 mg/Kg).

The concentration of sodium dithionite in samples was compared with FSANZ (Food Standard Australian New Zealand) by using T-test at confidence intervals of 95% (Table 2.). According to FSANZ, the maximum amount of sodium dithionite is 10 mg/kg (Te *et al.*, 2013). Figure 3. illustrates that the concentration of sodium dithionite in ginger, lemongrass, and bean sprouts exceeded the FSANZ standard.



#### IV. Conclusion

The present study revealed that sodium dithionite concentrations in ginger, lemongrass, and bean sprouts from Doeurm Kor Market, Phnom Penh, Cambodia, were above the maximum limits for sodium dithionite recommended by Food Standard Australian New Zealand (FSANZ). The use of sodium dithionite should be forbidden as it can pose a serious risk for consumers and rinsing or cooking vegetables before consuming are recommended.

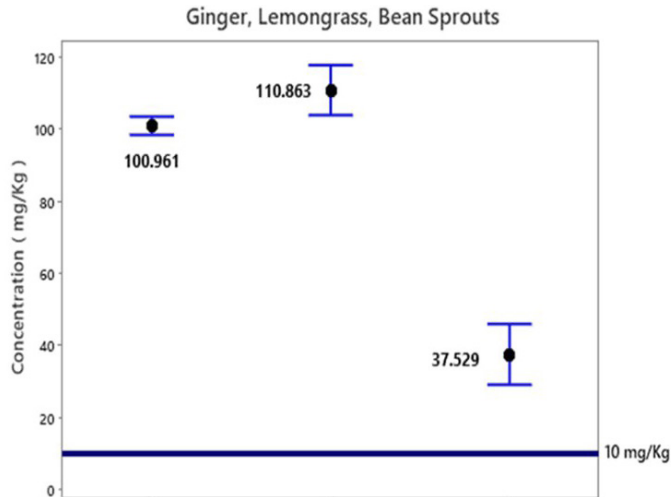


Figure 3. Comparison of Sodium Dithionite concentration in samples with Standard FSANZ (10 mg/Kg)

#### Reference:

- Chen, L., De Borba, B., & Rohrer, J. (2016). Determination of total and free sulfite in foods and beverages. *Thermo fisher scientific*, 54, 1-8.
- European Commission. (2002). Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official Journal of the European Communities*, 50, 8-36.
- Mirza Alizadeh, A., Mohseni, M., Zamani, A. A., & Kamali, K. (2015). Polarographic determination of sodium hydrosulfite residue (Dithionite) in sugar and loaf sugar. *Food analytical methods*, 8, 483-488.
- Nunn, R., Young, L., & Ni Mhurchu, C. (2021). Prevalence and types of non-nutritive sweeteners in the New Zealand food supply, 2013 and 2019. *Nutrients*, 13(9), 3228.
- Sila, S., & Navakhun, A. (2017). Simple method for determination of sodium hydrosulfite by spectrophotometric technique. *Burapha Science Journal*, 173-186.
- Te, M.K.K., Ahitereiria, M.A. (2013). Food Standards Australia New Zealand (FSANZ). Application A1088- Sodium Hydro-sulphite as a Food Additive, 7, 1-7.
- Yahia, E. M., & Carrillo-Lopez, A. (Eds.). (2018). *Postharvest physiology and biochemistry of fruits and vegetables*. Wood-head publishing.



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# 5

## Evaluation of Histamine Content in Lactic Fermented Fish Product, Nem by Enzymatic Test Kit

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### Highlight

- The study was conducted to determine and evaluate the histamine concentration in Cambodian lactic fermented fish, Nem collected from Battambang, Siem Reap, and Phnom Penh
- The histamine content was analyzed by using the colorimetric enzyme assay, Kikkoman Histamine Test Kit
- 95% of samples contained histamine less than maximum limit by European Union food safety regulation

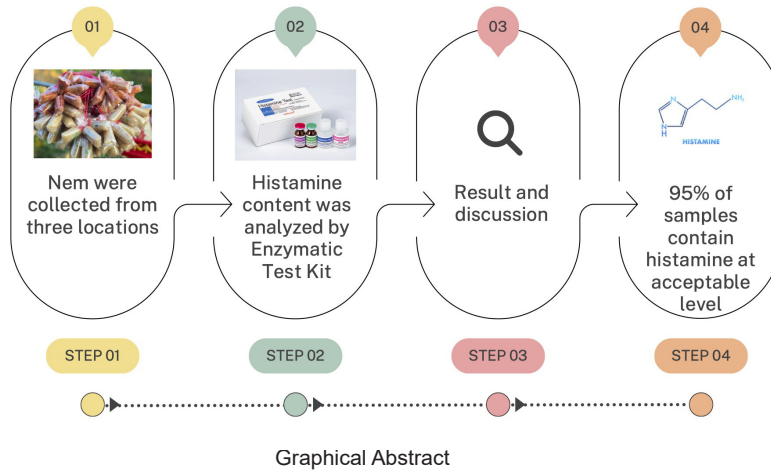


Fermented fish product and Nam

### I. Introduction

Nem is a popular appetizer produced from either minced fermented fish (Nem Trey and Sangvak) or minced fermented fish mixed with pork skin (Nem Sbek Chrouk). These products are packed with banana leaves or synthetic casing and need two to three days for fermentation (Nguyen *et al.*, 2010). Although these fish products are well-liked by consumers, questions about their safety are still the concerns. According to a scientific review by the European Food Safety Authority (EFSA) in 2011, biogenic amines found in foods, particularly fermented food, could be dangerous to the health of consumers (EFSA, 2011). Histamine (HIS) has been demonstrated to cause severe symptomatology including skin rashes, headaches, nausea, diarrhea, and changes in blood pressure (Botello-Morte *et al.*, 2022).

The production of histamine in food is involved with microorganisms' activity. The histamine is produced by oxidative decarboxylation of the precursor amino acid L-histidine, which is catalyzed by the L-histidine decarboxylase enzyme (Rachmawati & Triwibowo, 2022). Food storage condition, pH and salt amount are the main factors affecting the growth of microorganisms thus leading to the production of histamine (Botello-Morte *et al.*, 2022; Visciano *et al.*, 2020). Considering the safety of Nem products which are usually produced under improper or non-standardized conditions, this study collected Nem samples from three provinces and cities namely Battambang, Siem Reap, and Phnom Penh to analyze their histamine content using the enzymatic test kit.



## II. Material and Reagents

Chemicals used in this study include Histamine test kit (Kikkoman, Japan), Histamine dihydrochloride ( $\geq 99\%$ ) (Sigma-Aldrich, USA), EDTA 0.5M (Sigma-aldrich, USA), HCl (37%) (Scharlab, Germany). Standard stock solution of histamine concentration at 1000 ppm was prepared by dissolving of histamine dihydrochloride with 0.1 N HCl and adjusted to 25 mL.

## III. Sample Collection

Table 1 showed about the information on collected samples including sampling area, sample type, and sample code.

N°	Province	Sample type	Sample Code
1	Battambang (n=26)	Nem Trey (n=9)	BTB-NT
		Nem Sbek Chrouk (n=13)	BTB-NC
		Songvak (n=4)	BTB-SV
2	Siem Reap (n=6)	Nem Trey (n=4)	SR-NT
		Nem Sbek Chrouk (n=2)	SR-NC
3	Phnom Penh (n=29)	Nem Sbek Chrouk (n=19)	PP-NC
		Nem Trey (n=10)	PP-NT

#### IV. Histamine analysis

Initially, 10 g of sample was weighted and homogenized with 30 mL of pure water using homogenizer (25 digital Turrax, IKA, Korea) for 2 min at 8000 rpm. Then, four grams of homogenized sample was transferred into 50 mL centrifuge tube and 5 mL of 0.5M EDTA was added to the tube and brought to 25 mL by pure water. After that the sample was boiled for 20 min after mixing by vortex for 1 min to complete the dilution. Next, the tube was placed into ice box for cooling down to 15°C and was mixed by vortex for 30 s. Then, the tube was centrifuged for 5 min with 9500 rpm at 4°C and the extracted samples was collected by syringe filter (0.45 µm in pore size). The 0.5 mL of extracted sample was mixed with colorimetric reagent (tetrazolium salt and 1-methoxy PMS) and enzyme reagent (histamine dehydrogenase) in an eppendorf tube. The eppendorf was incubated at 37°C for 15 min and protected from sunlight by using aluminum foil. Finally, the absorbance measured with spectrophotometer (UV-Viz-1280, Shimadzu, Japan) with 1 cm cuvette and wavelength 470 nm and the histamine concentration in sample was calculated from calibration curve. The method verification was expressed as linearity, precision, and accuracy.

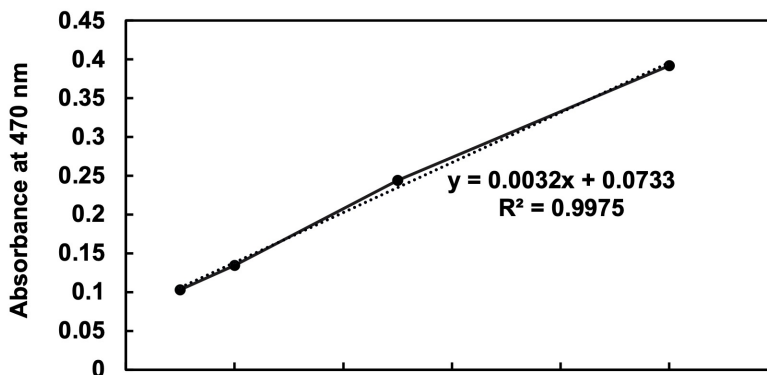


Figure 1. Calibration curve of histamine standard solution

#### V. Result and Discussion

Method verification

##### Linearity

The linear regression data for the calibration curves, which generated from measurement of absorbance of standard solution of histamine at concentration 10, 20, 50, and 100 ppm. Linearity equation was found  $y = 0.0032x + 0.0733$  and correction coefficient  $R^2 = 0.9975$  (Figure 1). The correction coefficient showed a comparable to the other reported, which have the correction coefficient at 0.999 (Jain *et al.*, 2011).

The limit of detection (LOD) and limit of qualification (LOQ) of histamine were determined by using standard deviation of response and slope. The LOD and LOQ represented in 0.04 ppm and 0.13 ppm, respectively.

##### Precision and Accuracy

The precision was reported as % relative standard deviation (%RSD). For this, 20 ppm concentration solution were measured three times in a day and the same was measured in the next 2 days. The recovery result generated by standard solution of histamine addition. In this experiment, the sample was spiked with 20 ppm histamine standard solution. The recovery rate was found to be 115.93 as mentioned in Table.2.

#### VI. Detection of histamine in Nem products

Table 3. showed the histamine content detected in BTB-NT, PP-NT, and SR-NT with the concentration

of 41.61±23.65 ppm, 45.36±17.71 ppm, and 90.22±64.95 ppm, respectively. While, NC contained HIS of 44.41±16.56, 105.97±45.34 and 27.47±18.83 ppm for samples collected from Battambang, Siem Reap, and Phnom Penh, respectively. Moreover, SV showed a lower HIS concentration 30.86±20.47 ppm. In summary, 58 of 61 samples were detected below permit level (<100ppm) based on EFSA which are considered safe (EFSA, 2011).

Table 2. Precision and accuracy

Concentration (ppm)	Precision				Accuracy		
	Intra-day (n=3)		Inter-day (n=2)		Detected concentration (ppm)	%RSD	Recovery (%)
	Detected concentration (ppm)	%RSD	Detected concentration (ppm)	%RSD			
20	20.96	1.72	21.17	1.39	23.19	0.78	115.93

Table 3. Concentration of histamine detected in Nem products

N°	Provinces	Type of products	Number of samples	Histamine (ppm)
				Mean±SD
1	Battambang	BTB-NC	13	44.41 <sup>a</sup> ±16.56
		BTB-NT	9	41.61 <sup>a</sup> ±23.65
		BTB-SV	4	30.86 <sup>a</sup> ±20.47
2	Phnom Penh	PP-NC	19	27.47 <sup>a</sup> ±18.83
		PP-NT	10	45.36 <sup>a</sup> ±17.71
3	Siem Reap	SR-NC	2	105.97 <sup>b</sup> ±45.34
		SR-NT	4	90.22 <sup>b</sup> ±64.95

Values with different superscript letters in the same column indicate significant differences at p-value < 0.05 by Turkey test. SD: standard deviation

The histamine content detected were statistically different for Nem samples collected in Siem Reap compared to Battambang and Phnom Penh. In general, the products produced traditionally may differ from one place to another with the processing techniques or hygiene of the surrounding environment.

Thus, the differences of histamine contents in these different locations may be due to the difference conditions in processing and storage of samples such as temperature control in pre-, during, post process, and transportation. As suggested by Gardini *et al.*, (2016), ability of histamine producing should be different based the impact of environmental condition on histamine formation. Additionally, formation of biogenic amines in fish is species specific based on histidine presented (Prester, 2011). So, this should be a factor influenced on formation of histamine. Trey Diep (*Channa micropellets*), Trey Noun Chan (*Chanos chanos*), Trey Trasok (*Probarbus labeaminor*), Trey Chhdor (*Giant snake head*), Trey Proul (*Cirrhinus microlepis*), Trey Kaek (*Labeo chrysophekadion*), or Trey Slat (*Notopterus notopterus*) are common fish species for Nem production according to the survey with producers.

## VII. Conclusion

Histamine content was analyzed in 61 samples of Nem products collected from Battambang (26 samples),

Siem Reap (6 samples), and Phnom Penh (29 samples). 95% of samples were detected below permit level (<100ppm) which are considered safe. Samples from Siem Reap were higher than other provinces which may be due to processing practice, origin of raw material, and storage of the final products. Further investigation on factor contributing to histamine production in different production sites should be conducted for better prevention measure in Nem production.

#### Reference:

- Botello-Morte, L., Moniente, M., Gil-Ramírez, Y., Virto, R., García-Gonzalo, D., & Pagán, R. (2022). Identification by means of molecular tools of the microbiota responsible for the formation of histamine accumulated in commercial cheeses in Spain. *Food Control*, 133, 1–10. <https://doi.org/10.1016/j.foodcont.2021.108595>
- EFSA. (2011). Scientific Opinion on risk based control of biogenic amine formation in fermented foods. *EFSA Journal*, 9(10), 1–93. <https://doi.org/10.2903/j.efsa.2011.2393>
- Gardini, F., Özogul, Y., Suzzi, G., Tabanelli, G., & Özogul, F. (2016). Technological factors affecting biogenic amine content in foods: A review. *Frontiers in Microbiology*, 7(8), 1–18. <https://doi.org/10.3389/fmicb.2016.01218>
- Jain, P. S., Chaudhari, A. J., Patel, S. A., Patel, Z. N., & Patel, D. T. (2011). Development and validation of the UV-spectrophotometric method for determination of terbinafine hydrochloride in bulk and in formulation. *Pharmaceutical Methods*, 2(3), 198–202. <https://doi.org/10.4103/2229-4708.90364>
- Nguyen, H. T. H., Elegado, F. B., Librojo-Basilio, N. T., Mabesa, R. C., & Dizon, E. I. (2010). Isolation and characterisation of selected lactic acid bacteria for improved processing of Nem chua, a traditional fermented meat from Vietnam. *Beneficial Microbes*, 1(1), 67–74. <https://doi.org/10.3920/BM2009.0001>
- Prester, L. (2011). Biogenic amines in fish, fish products and shellfish: a review. *Food Additives and Contaminants - Part A*, 28(11), 1547–1560. <https://doi.org/10.1080/19440049.2011.600728>
- Rachmawati, N., & Triwibowo, R. (2022). Histamine fish poisoning (HFP) in Indonesia: Current status and challenges. *E3S Web of Conferences*, 344, 1–14. <https://doi.org/10.1051/e3sconf/202234405001>
- Visciano, P., Schirone, M., & Paparella, A. (2020). An overview of histamine and other biogenic amines in fish and fish products. *Foods*, 9(12). <https://doi.org/10.3390/foods9121795>



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# 6

## Bayesian Updating of the Seismic Behavior of Nuclear Reinforced Concrete Structure Application to SMART2013 Mock-Up

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### Highlight

- The paper presents the technic of Bayesian updating on Young's modulus and damping ratios of concrete structures for obtaining a good estimation of eigenfrequencies and acceleration response spectra
- The paper presents the technic of using meta-modeling while performing Bayesian updating through Markov Chain Monte Carlo (MCMC)
- The Bayesian technique and meta-modeling are applied for an elastic equivalent simulation with different seismic intensities of the Northridge signal
- The updated Young's modulus of concrete has been reduced logically as the observation of cracks from the experimental point of view from the SMART 2013 mock-up placed on a shaking table (AZALEE/CEA)
- The second level of updated parameters is a damping ratio, the updated values provided a good amplitude of acceleration response spectra between the results of elastic equivalent simulation and the experiments

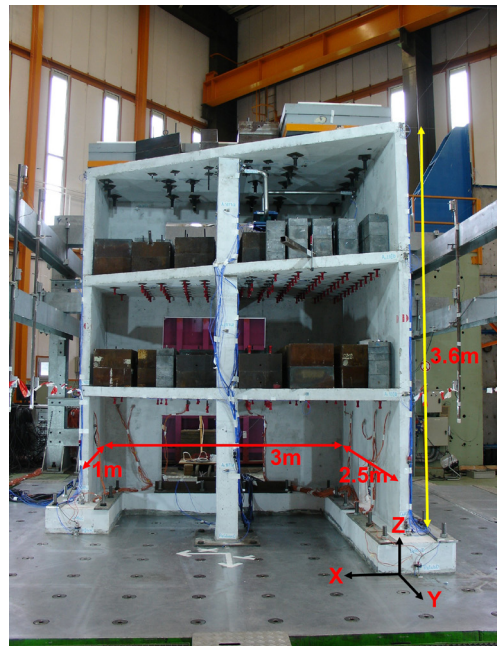
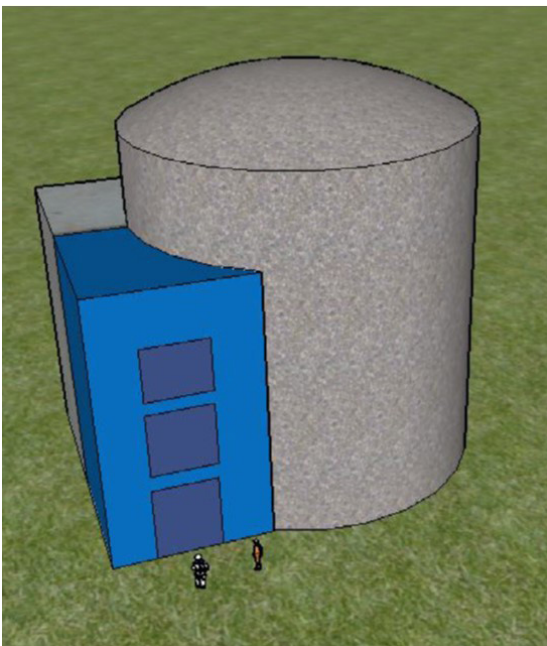


Figure 1. global view of electricity building and SMART 2013 mock-up on shaking table



## I. Introduction

In this paper, the Bayesian technique is applied to the mechanical behavior of structural elements, such as reinforced concrete structures. The sources of uncertainties come from the poor knowledge of the input parameters (constitutive law of the material) and the epistemic phenomenon in numerical modeling (boundary conditions). The objective is to develop a methodology based on the Bayesian approach and the available measurements from in situ, so as to update the current state of parameters for the constitutive law of the material. Afterward, the new numerical simulation could be launched with the initial state based on the updated parameters in order to understand if the structure remains sufficient strength for respecting the safety criteria.

The Bayesian updating technique is popularly used in various domains. There exist in the literatures, (Richard B. A., 2012; Rossat D. B.-P., 2022; Rossat D. B.-M., 2021; Tekeste, 2022), where the Bayesian is applied to different purposes, such as to estimate the fragility curve by updating the structural capacity; to forecast the leakage rate of concrete containment building. In the framework of METIS project (METIS, 2020), the Bayesian approach is considered for the SMART 2013 mock-up (Richard B. C.-E., 2016; Richard B. M., 2015; Belletti, 2017). Initially, the elastic equivalent simulation is proposed, and the task is to update the effective rigidity of the structure (ex: Young's modulus; damping ratio) based on the recorded eigenfrequencies; acceleration, and cracking pattern.

The SMART 2013 mock-up (CEA, 2013) is initiated from an electricity building that is attached to the nuclear containment building, see Figure 1. As mentioned, SMART 2013 mock-up is a reduced scale of a reinforced concrete building at a factor of 4. In Figure 1, the SMART 2013 mock-up is placed on the shaking table, this mock-up is composed of walls; slabs; beams; foundation, and column to assure stability during the test. The mock-up is well monitored, where displacements and accelerations are recorded during the experimental test. The seismic excitation is realized through the shaking table in two horizontal directions. Three seismic sequences are considered at different peak ground acceleration (PGA), they are the design level (0.2g); Northridge main-shock (1.78g), and Northridge after-shock (0.37g).

## II. Application of Bayesian updating on SMART mock-up

In Figure 2, the methodology of the application of Bayesian updating to the SMART 2013 mock-up is illustrated. The scripts of numerical simulation are developed on the software, Cast3M (Cast3M, 2019). As testing, a classical computation of eigenfrequencies and a full elastic analysis of the base model cost about 2 minutes and 6 minutes, respectively. In consequence, the calling of Markov Chain Monte Carlo (MCMC=1E5 computations) during the Bayesian updating would be a time-consuming process. To overcome this issue, the construction of a Meta-model (Berveiller, 2012; Marelli, 2022) is proposed. In e.q 1, the Meta-model is considered as a mathematic function and is used to replace the complex numerical simulation, where X is the vector of the input parameters and Y is the vector of quantities of interest.

$$Y=M(x,t) \qquad \text{e.q 1}$$

In this paper, the Meta-model is based on the polynomial chaos expansion (PCE) equations (Blatman, 2011). Thus, about 8000 numerical simulations (around 4 days) are launched and mean to identify the coefficients of PCE for constructing the Meta-model.

Afterward, the method of Bayesian with the MCMC approach is applied in order to update the input parameters,

which is obtained from the posterior distribution:

$$\pi(\theta|x) = \frac{\pi(x|\theta)\pi(\theta)}{\pi(x)} \quad \text{e.q 2}$$

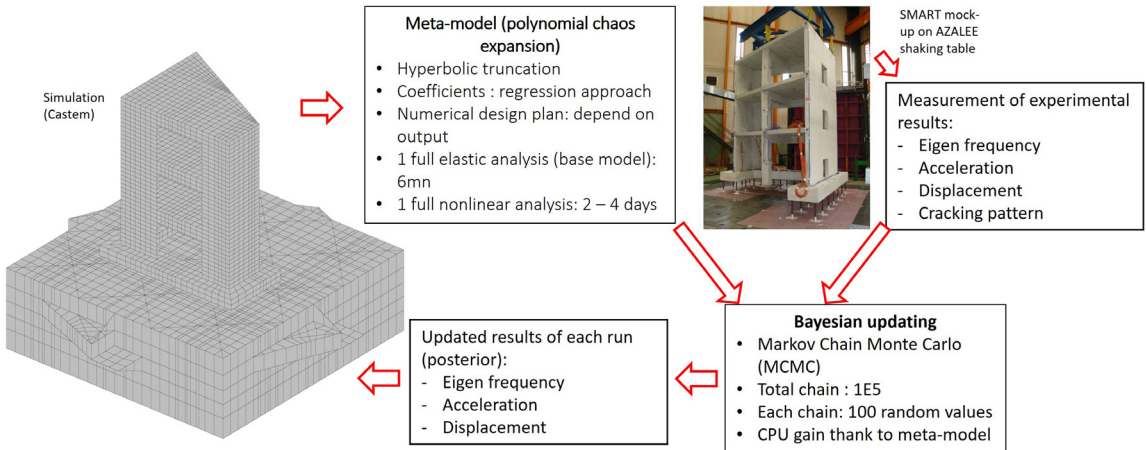


Figure 2. Methodology of Bayesian on SMART 2013 mock-up

However, the literatures provided in the introduction seeks to detail the equations for the Bayesian inference technic. In practice, the MCMC with the Random Walk Metropolis-Hastings (RW-MH) algorithm (Hastings, 1970; Metropolis, 2004) is widely used for solving inverse problems. As illustrated in Figure 2, the MCMC would call directly to the Meta-model instead of re-launching the numerical simulation. The statistic properties of the posterior distribution are used for the exact finite element simulation in order to verify the estimation of eigenfrequencies and acceleration.

It is worth mentioning that the Bayesian updating is realized in two phases successively: updating Young's modulus for eigenfrequencies and updating damping ratios for the amplitude of acceleration spectra.

### III. 1st Bayesian Updating for Eigenfrequencies

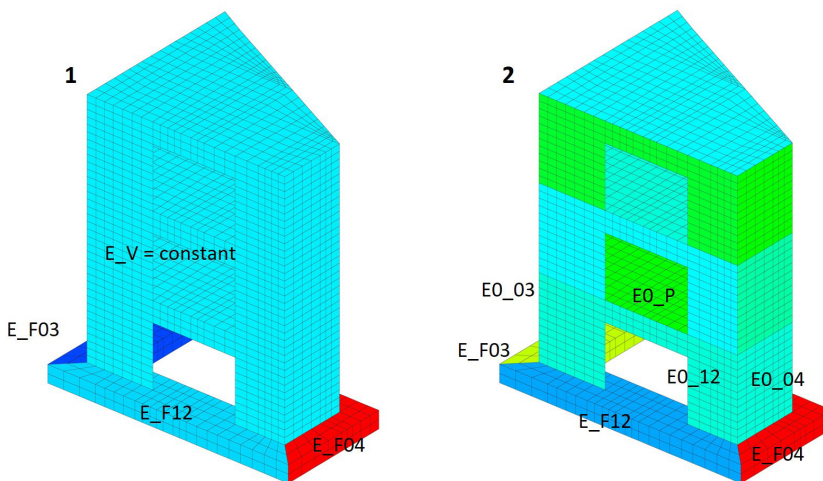


Figure 3. Configuration of 1st Bayesian updating

In Figure 3, two configurations of Bayesian updating of concrete Young's modulus for obtaining a good estimation of eigenfrequencies are proposed. The first configuration, only three Young's modulus in the foundation are activated for Bayesian updating. This configuration corresponds to the elastic runs, which refer to seismic sequence at the design level (0.2g), and no crack is observed during the experiment. The second configuration is applied to the non-linear runs where Young's modulus of the upper structure is possible to be activated in accordance with a different crack observation from the experiment. Furthermore, a global Meta-model is constructed for the 1st Bayesian updating.

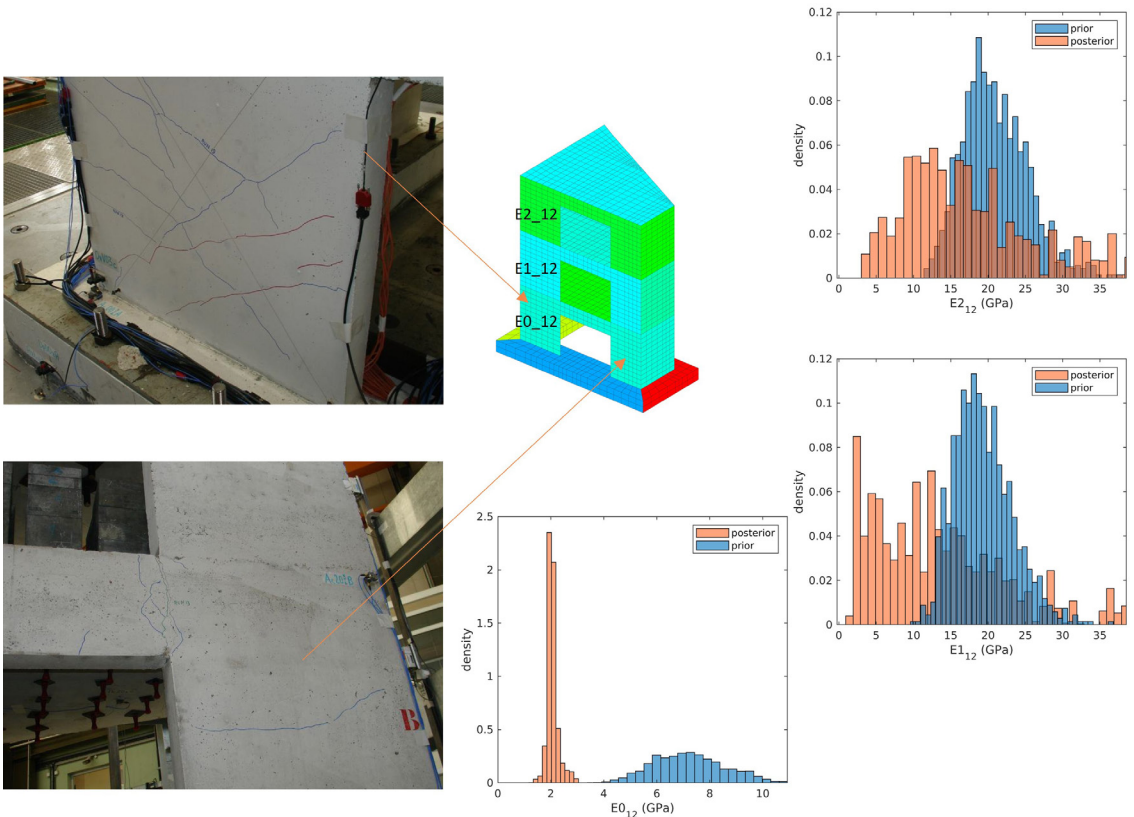


Figure 4. Bayesian updating of Young's modulus of the wall at run #19 (PGA=1.1g)

Figure 4 presents directly the results of final run #19, where the measured PGA on the shaking table reaches 1.1g. As explained, the decision to activate updating Young's modulus depends on crack observation at different structural elements obtained from the experiment. Furthermore, Young's modulus is updated successively from run to run (elastic to non-linear seismic sequences). It is seen in Figure 4 and Figure 5, that once the cracks are developed, the posterior distribution shift to the left, which means that Young's modulus reduce. In Figure 5, the comparison of eigenfrequencies among the posterior; prior, and experiment is illustrated as well. The posterior properties have improved the estimation of the eigenfrequency.

#### IV. 2nd Bayesian Updating for Acceleration Response Spectra

The second Bayesian updating means to update the damping ratios in order to obtain a good estimation of the acceleration response spectra. The Meta-model, which is served for this Bayesian updating, is reconstructed based on the previous posterior properties of the updated Young modulus. Besides that, the damping model

used in the numerical simulation is achieved by the Rayleigh formulation and is covered between the first and third eigenfrequencies ( $f_1$ ;  $f_3$ ).

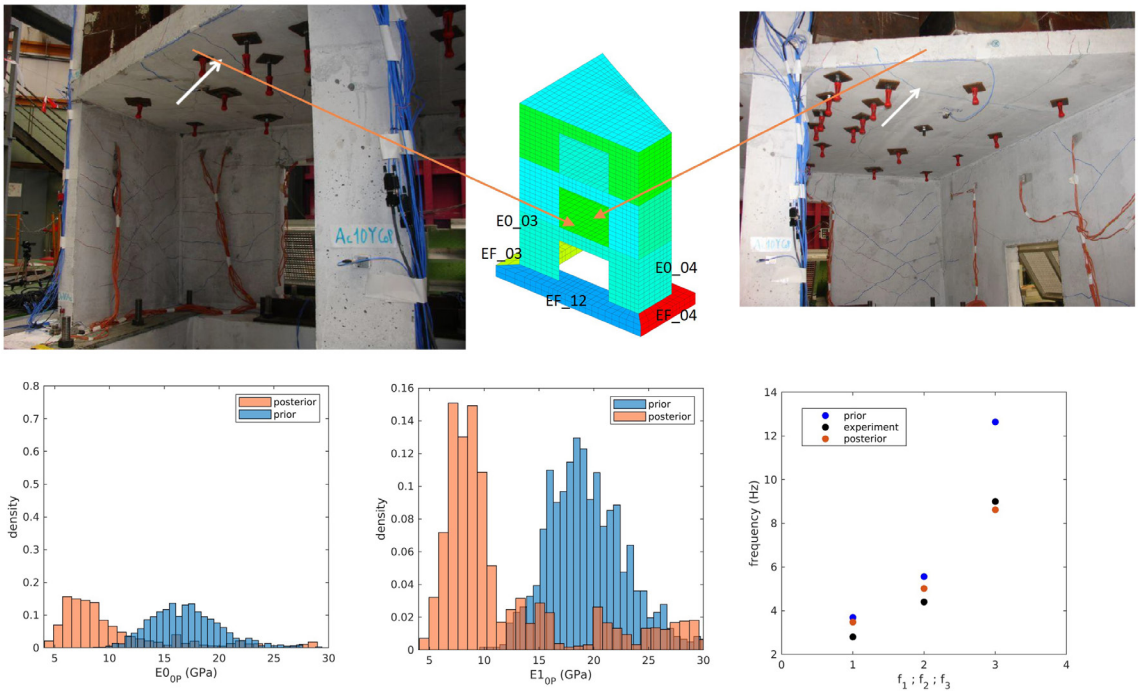


Figure 5. Bayesian updating of Young's modulus of the slab at run #19 (PGA=1.1g)

Figure 6 shown the posterior distribution of the updated damping ratio for run #19. It is seen that the form of the distribution is transformed from the supposed uniform distribution prior to a normal distribution for the posterior. The comparison has been made at the control point D, and the acceleration response spectra obtained through the dynamic simulation with all the updated posterior properties give a good agreement with those of the experiment in both, X and Y directions.

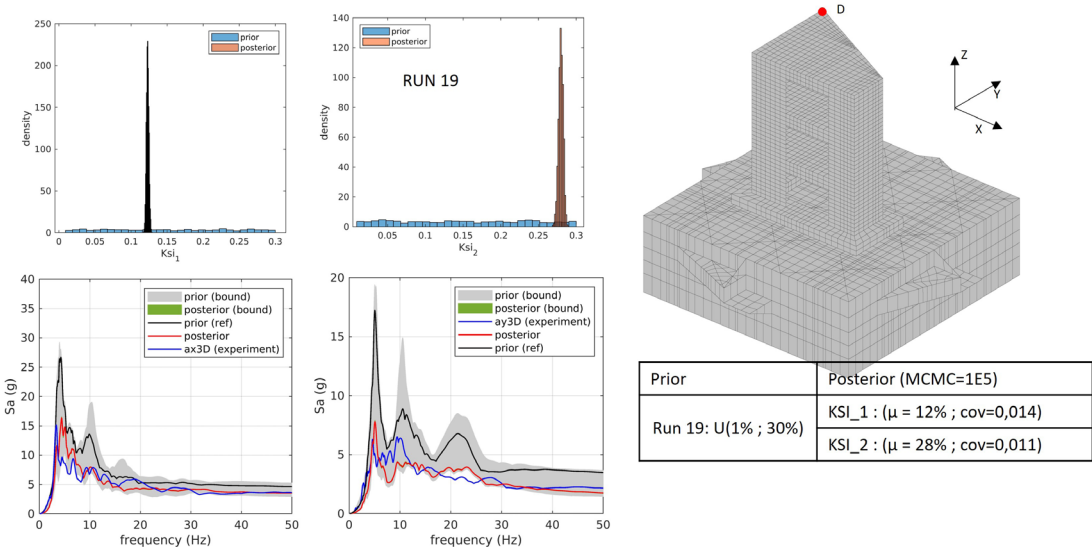


Figure 6: Bayesian updating of damping ratios for the acceleration response spectra (5% of natural damping ratio)

## V. Conclusion

In this paper, the methodology of Bayesian updating for the elastic equivalent simulation has been developed into two phases and is applied to the SMART 2013 mock-up. The Bayesian updating is achieved through the MCMC approach and thanks to the Meta-model that has largely reduced the computation time. The first Bayesian updating is to update Young's modulus for estimating a good eigenfrequencies and to shift horizontally the curve of acceleration response spectra. The second level of Bayesian updating is to figure out the posterior properties of the damping ratios of the structure in order to obtain a good amplitude at the peak of the acceleration response spectra. The results presented in this paper demonstrate the robustness of the proposed methodology. However, the remaining tasks are to reconstruct the fragility curve with the updated posterior properties, which is an important factor for identifying the safety criteria of the structure.

## Reference:

- Belletti, B. S. (2017). Validation of the parc-cl 2.0 crack model for the assessment of the nonlinear behaviour of RC structures subjected to seismic action: Smart 2013 shaking table test simulation. *Engineering Structures*, 759-773.
- Berveiller, M. L. (2012). Updating the long-term creep strains in concrete containment vessels by using markov chain monte carlo simulation and polynomial chaos expansion. *Structure and Infrastructure Engineering*, 425-440.
- Blatman, G. &. (2011). Adaptive sparse polynomial chaos expansion based on least angle regression. *Journal of Computational Physics*, 2345-2367.
- Cast3M. (2019). Code de Calcul aux Eléments Finis CAST3M. Technical Report Commissariat à l'Energie Atomique, CEA-DES/DM2S/SEMT.
- CEA, T. A. (2013). <http://data-tamaris.fr/>.
- Hastings, W. (1970). Monte carlo sampling methods using markov chains and their applications. *SCOPUS*, 97-109.
- Marelli, S. L. (2022). Technical Report Chair of Risk, Safety and Uncertainty Quantification, ETH Zurich, Switzerland. UQLab user manual – Polynomial chaos expansions.
- METIS, S. r. (2020). <https://metis-h2020.eu/>.
- Metropolis, N. R. (2004). Equation of State Calculations by Fast Computing Machines. *The Journal of Chemical*, 1087-1092.
- Richard, B. A. (2012). A methodology for robust updating of nonlinear structural models. *Engineering Structures*, 356-372.
- Richard, B. C.-E. (2016). Smart 2013: Experimental and numerical assessment of the dynamic behavior by shaking table tests of an asymmetrical reinforced concrete structure subjected to high intensity ground motions. *Engineering Structures*, 99-116.
- Richard, B. M. (2015). Smart 2008: Shaking table tests on an asymmetrical reinforced concrete structure and seismic margins assessment. *Engineering Structures*, 48-61.
- Rossat, D. B.-M. (2021). A bayesian strategy for forecasting the leakage rate of concrete containment buildings – application to nuclear containment buildings. *Nuclear Engineering and Design*, 111184.
- Rossat, D. B.-P. (2022). Bayesian updating for nuclear containment buildings using both mechanical and hydraulic monitoring data. *Engineering Structures*, 114294.
- Tekeste, G. C. (2022). Bayesian updating of seismic fragility curves through experimental tests. *Bulletin of Earthquake Engineering*.
- Wang, Z. Z. (2018). A Bayesian framework for estimating fragility curves based on seismic damage data and numerical simulations by adaptive neural networks. *Nuclear Engineering and*, 232-246.



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# TECHNOLOGY TRENDS

# Arbuscular Mycorrhizal Fungi as a Potential Biofertilizer for Agricultural Sustainability in Cambodia

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## Highlight

- AMF are soil fungi available as natural or inoculum products
- AMF are considered as a biofertilizer due to its nutrient (P, N) uptake
- Using AMF is a promising solution for sustainable agriculture in Cambodia
- More research is needed to identify AMF species and optimize inoculation techniques

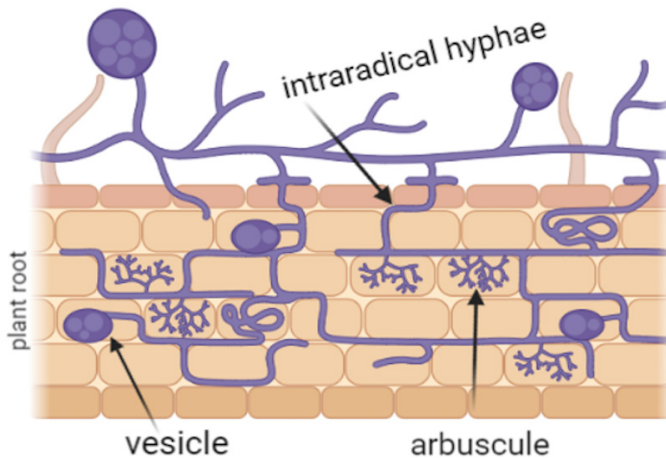


Figure 1 . Mycorrhizal structures inside the plant root.

## I. Introduction

In agriculture, AMF can enhance crop growth and yield by improving nutrient uptake, water use efficiency, and disease resistance, reducing the need for chemical fertilizers and pesticides. AMF also aid in restoring degraded agroecosystems by improving soil quality and plant establishment. Moreover, AMF has been found to contribute to soil carbon sequestration, which can help mitigate the impact of climate change (Bennett & Groten, 2022). Despite these benefits, there are currently no scientific reports regarding the application and availability of AMF in Cambodia. Thus, this article aims to introduce AMF and its biofertilizer potential as a trending technology that could help enhance agricultural sustainability in Cambodia.

## II. AMF as a biofertilizer

The escalating global demand for food, coupled with the need for environmentally responsible agricultural practices, has prompted a search for innovative solutions to boost crop yields while reducing the negative impacts of conventional farming. AMF have emerged as a biofertilizer because they could offer a sustainable approach to improve plant nutrient acquisition, enhance soil fertility, and minimize synthetic fertilizers (Ebbisa,



2022). It can be available as natural in the soil or inoculant products.

The key nutrient uptake by AMF is P, which is mainly available in the soil but cannot be directly uptake by the plant. AMF help P uptake by using the hyphae to explore and convert it to the available form that the plant can absorb. Once absorbed by AMF, P is transported via phosphate transporters to the arbuscule, where it is released into the plant cells (Figure 3). AMF can also facilitate N acquisition and other nutrients (Smith & Smith, 2011). AMF can contribute up to 80% of plant P and N (van Der Heijden *et al.*, 2015). By improving nutrient availability and altering root architecture, AMF can help plants withstand abiotic stresses, such as drought (Augé, 2001). These benefits make AMF a promising tool for sustainable and efficient agriculture.

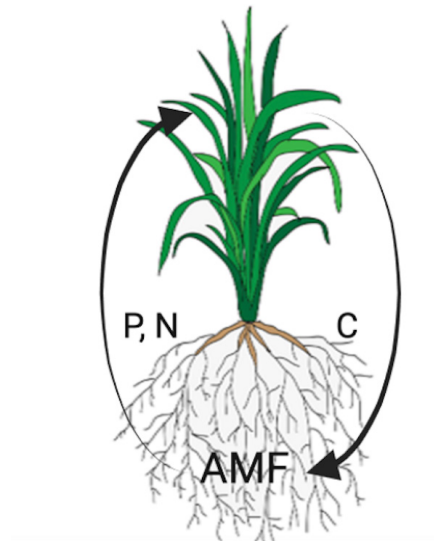


Figure 2. Mycorrhizal exchange of nutrient (N, P) uptake with plant sugar (C)

### III. AMF for agricultural sustainability in Cambodia

Cambodia's agriculture sector is vital to its economy and livelihoods, with most of the population engaged in farming, but the country faces with issues like soil degradation, low agricultural productivity, and vulnerability to climate change (ADB, 2021). For example, soil degradation, caused by intensive rice monoculture and the overuse of synthetic fertilizers and pesticides, has led to soil erosion and nutrient depletion, which poses a significant threat to the long-term sustainability of agriculture in the country. Additionally, Cambodia is vulnerable to the impacts of climate change, including erratic rainfall patterns, droughts, and floods, which can adversely affect crop production (ADB, 2021). AMF offer a sustainable and eco-friendly solution to address these challenges while improving crop yields and reducing environmental impact.

Rice is Cambodia's staple crop (ADB, 2021), and studies have shown that AMF can significantly increase rice yields while reducing fertilizer use (Mbodj *et al.*, 2018). In addition, key cash crops like cassava (Séry *et al.*, 2016) and rubber (Herrmann *et al.*, 2015) can also benefit from AMF associations, improving the income of smallholder farmers. AMF also play a crucial role in sustainable land management by improving soil structure and reducing erosion (Sosa-Hernández *et al.*, 2019). Therefore, integrating AMF into agricultural practices has shown promise in creating a more sustainable agricultural system. However, there are no scientific reports regarding the application and/or development of either natural or inoculum products in Cambodia, which requires more research, development, and dissemination among stakeholders.

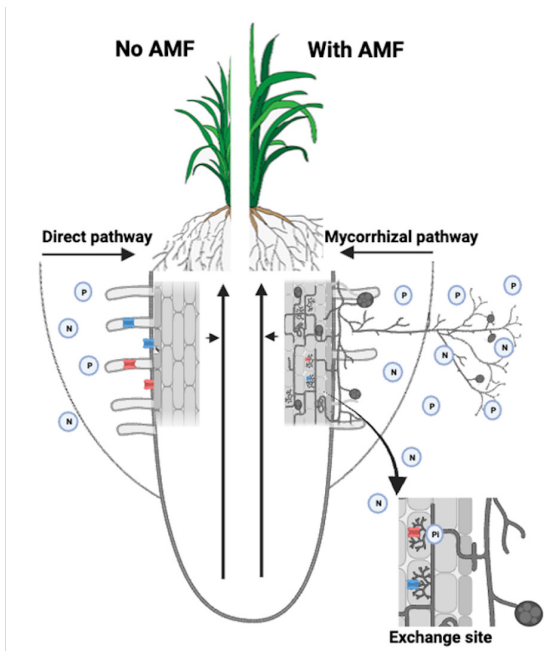


Figure 3. Plant with AMF use mycorrhizal pathway to uptake nutrient (P) in addition to direct uptake pathway compared to plant with no AMF

#### IV. Concluding Remarks

In conclusion, AMF have the potential to transform Cambodian agriculture by assisting with nutrient acquisition, reducing the dependence on synthetic fertilizers, and improving soil health. As Cambodia strives for agricultural sustainability to meet the needs of its growing population and organic reputation, integrating AMF into farming practices offers a promising solution, but actions need to be taken. One action is to raise awareness among farmers about the benefits of AMF and how to incorporate them into their farming practices. Also, more research is needed to identify local AMF species best suited to Cambodian soils and crops and optimize inoculation techniques to achieve this. Last, government policies encouraging sustainable farming practices, including using AMF, can provide the necessary framework for widespread adoption.

#### Reference:

ADB. (2021). Cambodia agriculture, natural resources, and rural development sector assessment, strategy, and road map. Manila, Philippines.

Augé, R. M. (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*, 11(1), 3-42.

Bennett, A. E., & Groten, K. (2022). The costs and benefits of plant–arbuscular mycorrhizal fungal interactions. *Annual Review of Plant Biology*, 73, 649-672.

Ebbisa, A. (2022). Arbuscular mycorrhizal fungi (AMF) in optimizing nutrient bioavailability and reducing agrochemicals for maintaining sustainable agroecosystems. In *Mycorrhiza-New Insights*. IntechOpen.

Herrmann, L., Bräu, L., Robin, A., Robain, H., Wiryakitnateekul, W., & Lesueur, D. (2016). High colonization by native arbuscular mycorrhizal fungi (AMF) of rubber trees in small-holder plantations on low fertility soils in North East Thailand. *Archives of Agronomy and Soil Science*, 62(7), 1041-1048.

Mbodj, D., Effa-Effa, B., Kane, A., Manneh, B., Gantet, P., Laplaze, L., ... & Grondin, A. (2018). Arbuscular mycorrhizal symbiosis in rice: establishment, environmental control and impact on plant growth and resistance to abiotic stresses. *Rhizosphere*, 8, 12-26.

Phoura, Y., Ohtomo, R., Nakanishi, H., & Kamoshita, A. (2023). Effects of arbuscular mycorrhizal fungi inoculation on infection and growth of rice and pearl millet in upland fields with three water regimes. *Plant Production Science*.

Séry, D. J. M., Kouadjo, Z. C., Voko, B. R., & Zeze, A. (2016). Selecting native arbuscular mycorrhizal fungi to promote cassava growth and increase yield under field conditions. *Frontiers in Microbiology*, 7, 2063.

Smith, S.E., & Smith, F.A. (2011). Roles of Arbuscular Mycorrhizas in Plant Nutrition and Growth: New Paradigms from Cellular to Ecosystem Scales. *Annual Review of Plant Biology*, 62, 227-250.

Sosa-Hernández, M. A., Leifheit, E. F., Ingrassia, R., & Rillig, M. C. (2019). Subsoil arbuscular mycorrhizal fungi for sustainability and climate-smart agriculture: a solution right under our feet?. *Frontiers in Microbiology*, 10, 744.

van Der Heijden, M. G., Martin, F. M., Selosse, M. A., & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist*, 205(4), 1406-1423.



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# 8

## AMRIT: An Affordable and Simple Water Purification Technology for Iron and Arsenic Removal Developed by IIT Madras

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Prof. T. Pradeep, Institute Professor, Department of Chemistry Indian Institute of Technology Madras Chennai, India

### Highlight

- The Anion and Metal Removal by Indian Technology (AMRIT) is a nanotechnology-based affordable and advanced solution for providing clean drinking water in areas affected by iron, arsenic, and fluoride
- The technology uses nanoscale adsorbents integrated into a filtration unit capable of removing a wide range of groundwater pollutants to obtain drinking water that conforms to international standards
- AMRIT is highly effective at removing arsenic from water, capable of handling up to 5 ppm of either forms of arsenic, and is at least 5-6 times more efficient than other adsorbents currently available
- The cost-effectiveness, higher efficiency, easy maintenance, and environmentally-friendly features make AMRIT an attractive technology compared to other solutions worldwide

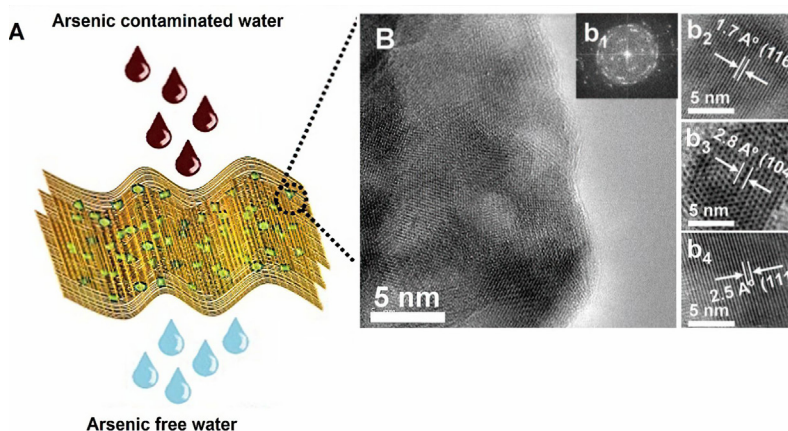


Figure 1. (A) Schematic of a filtration using nanomaterials. Contaminated water is passed through nanomaterials. (B) High resolution images of the particles showing nanometer size particles

## I. Introduction

Anion and Metal Removal by Indian Technology called AMRIT developed by Prof. T. Pradeep and his team at the Indian Institute of Technology Madras, India is an affordable and advanced solution for providing clean drinking water in iron, arsenic, and fluoride-affected areas of the world. This utilizes

regions of India, using different models, ranging from small household units of 10 litres a day to large scale community units of millions of litres per day. These materials-based solutions are green and sustainable. The cost-effectiveness, higher efficiency, easy maintenance and environmentally friendly features make AMRIT an extremely attractive technology

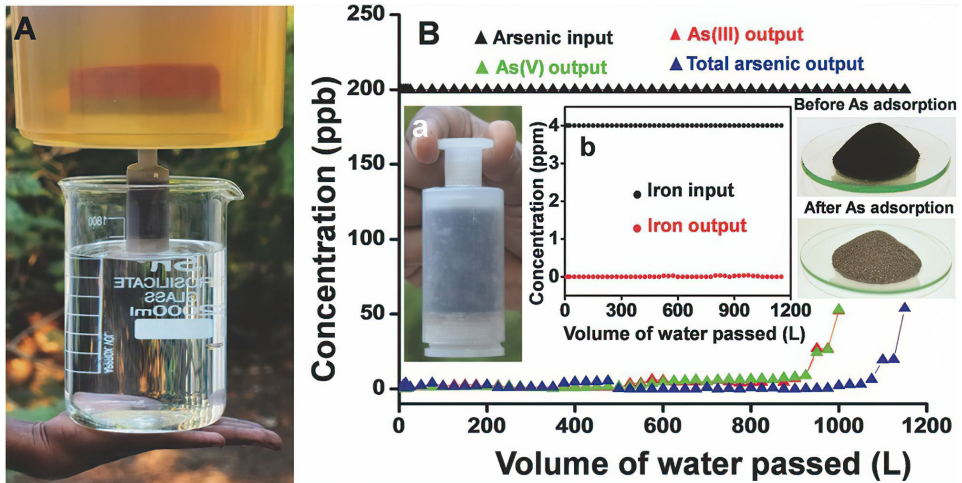


Figure 2. Set-up used for laboratory scale filtration of arsenic ( $\text{As}^{3+}$  and  $\text{As}^{5+}$ ) and iron ( $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$ ) contaminated water using a cartridge containing AMRIT materials fitted with a porous clay prefilter. B) Arsenic concentration in the water using a 60 g cartridge with the input as shown in A), inset (a) is the cartridge having 20 g adsorbent, inset (b) is iron output for the same input. The photographs of granular material before (black) and after (brown) arsenic adsorption are in the inset.

nanotechnology. It uses nanoscale adsorbents integrated into a filtration unit capable of removing a wide range of groundwater pollutants to obtain drinking water conforming to international standards.

The AMRIT system operates by passing raw water through a composite filtration unit, which removes contaminants such as anions such as arsenate and arsenite as well as heavy metals such as uranium, chromium, etc., in the form of their ions, making the treated water safe for human consumption. With its higher efficiency, the AMRIT purification process guarantees that the treated water conforms to international safety standards. This technology has been widely implemented as affordable point-of-use water purifiers in the worst arsenic and iron-affected

when compared to other solutions presently available worldwide.

Materials used in AMRIT are prepared by an exceptionally simple procedure using water-based synthesis at room temperature. The synthesized materials are insoluble in water and the synthesis is similar to biological processes. The materials employed in this technology have been engineered to enhance contaminant adsorption capacity, and the nanoparticles are tightly bound in a matrix to prevent contaminant leaching. These materials are made with abundantly available raw materials with zero use of non-biodegradable materials. To the naked eye, the material appears similar to bulk material; however, upon closer examination,

nanoscale features become visible (Figure 1). The material is comprised of a bio-organic network that traps nanoparticles within it.

Older technologies for similar applications used activated alumina for the removal of arsenic and

vis activated alumina and 10 times more effective vis-à-vis iron hydroxide. With the advent of new technologies, water purification plants can become smaller and more cost-effective.

## II. Performance and affordability in terms of Iron Removal



Figure 3. Photograph of AMRIT community unit installed in the state of Punjab, India. Oxidizer vessel through which air is pumped into the water for oxidation of Fe(II) to Fe(III), (B) Iron removal vessel for removing colloidal iron, (C) Arsenic removal vessel for removing arsenic (III and V) and (D) Polisher vessel for removing any foul smell or other impurities from the purified water. Microbial disinfection is also implemented if needed. The units are also monitored online with sensors for water quality and quantity. Implementations have been done by Hydromaterials Pvt. Ltd. (Detailed implementation of community units implemented in India can be accessed through the link: <https://pradeepresearch.org/towards-an-arsenic-free-punjab-2/>)

fluoride from the drinking water and its adsorption capacity was extremely poor. As a result, huge quantities of activated alumina were used. During the purification process, some traces of alumina were also released into the drinking water and its prolonged consumption results in neurological disorders. In addition, the sludge obtained from the purification process using activated alumina causes re-contamination to the environment. The AMRIT composition is over 25 times more effective vis-à-

The AMRIT system is capable of effectively treating water with high concentrations of colloidal iron up to 50 ppm, bringing the output well below the limit of <100 ppb, without the need for backwash. The AMRIT system has superior adsorption kinetics and enables efficient iron removal with minimal contact time, resulting in reduced treatment costs, smaller filtration units, and low-pressure operation with fewer maintenance requirements. This method of iron removal in the AMRIT unit is first-of-its-kind in the

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world and is a near-zero maintenance system. The unit has an in-built mechanism for indicating its life.

### **III. Performance and affordability in terms of Arsenic Removal**

The AMRIT composition is highly effective at removing arsenic from water, capable of handling up to 5 ppm of either form of arsenic (As<sup>3+</sup> and As<sup>5+</sup>) or both and reducing the output to the level below the detection limit (Figure 2). Compared to other adsorbents currently available, it is at least 5-6 times more efficient. Furthermore, the contact time required for the removal of arsenic is less than a minute, and the composition can be used in small 0.2 mm sizes, resulting in negligible pressure drop. This would offer several benefits, including reduced treatment costs, smaller filtration units, and the ability to operate the unit with minimal pressure, which can be easily maintained by local communities and reduces the amount of sludge produced. Prof. Pradeep and his team have implemented this technology as an affordable and sustainable point-of-use water purifier in many regions of India that are severely affected by iron and arsenic contamination (<https://pradeepresearch.org/towards-an-arsenic-free-punjab-2/>) (Figure 3). This Indian-proven technology now provides arsenic and iron-free water to over 1.3 million people daily. With over 2000 community installations and 25,000 home units distributed nationwide, the technology has proven to be affordable, sustainable, and scalable. Successful installations across India suggest that the technology can address any water quality situation as India is a large nation with varying water quality. It makes one confident of tackling similar challenges anywhere in the world.

AMRIT provides clean water at a filtration rate of 25,000 to 2,000,000 litres per day, at a cost lower than USD 0.03 per litre, including all equipment, consumables, and maintenance expenses, assuming a 10-year operational period for the plants. Home units come at a different cost. The AMRIT service is available from home units to community units.

In our outreach program, the International Centre for Clean Water (ICCW), IIT Madras, India in collaboration with the Rural Water Sanitation Team (RWST), an organization working in the WASH program in Cambodia conducted several trials of AMRIT household (HH) units in various regions of Cambodia, including Kandal province where the arsenic concentration exceeds 500 ppb. The analysis of the output water from the AMRIT HH unit demonstrated that the arsenic level was reduced well below 10 ppb, which is considered the safe limit for human consumption by WHO. As part of our initiative to promote access to safe drinking water, we have distributed more than 300 HH units in various regions of Cambodia. Considering the large population, the AMRIT community unit could be a good choice to supply purified water from 25 KLD (Kilo litre per day) to 1 MLD (million litre per day) depending on the demand. The AMRIT community unit is intended for online use, where water from the bore well is treated and stored in an overhead reservoir. The treated water then flows through the existing distribution network under gravity.

Our objective is to implement the large-scale AMRIT community units in Cambodia. This move is expected to have a substantial impact on enhancing the quality of drinking water available to local communities and, consequently, promoting the general health and well-being of the local population.

Recently, Prof. Pradeep was awarded the VinFuture Special Prize dedicated to Innovators from Developing Countries for his development of a low-cost filtration system to remove arsenic and other heavy metals from

drinking water. This ground-breaking technology has received other prestigious awards worldwide, such as the Nikkei Asia Prize and the Prince Sultan bin Abdul Aziz International Prize for Water demonstrating its exceptional achievements and widespread recognition. Full details are available on the link, <https://pradeepresearch.org/news/general-highlights/>.

In conclusion, the AMRIT system is a ground-breaking solution to address the challenge of providing safe drinking water in areas where groundwater contamination is a concern. Its innovative approach to water purification has the potential to revolutionize the field of water treatment, thereby improving the quality of life for millions of people globally.

#### Reference:

Kumar, A. A., Som, A., Longo, P., Sudhakar, C., Bhui, R. G., Gupta, S. S., Anshup, M. U. S., Chaudhary, A., Kumar, R., Pradeep, T., Confined Metastable 2-Line Ferrihydrite for Affordable Point-of-Use Arsenic-Free Drinking Water, *Adv. Mater.*, 2017, 29, 1604260.

Sankar, M.U., Aigal S., Maliyekkal, S.M., Chaudhary, A., Anshup, M. U. S., Kumar, A.A., Chaudhari, K., Pradeep, T. (2013) Biopolymer-reinforced synthetic granular nanocomposites for affordable point-of-use water purification. *PNAS*, 2013, 110, 8459–8464.

Pradeep, T., Noble metal nanoparticles for water purification: a critical review, *Thin Solid Films*, 2009, 517, 6441-6478.

Pradeep, T., *A Textbook of Nanoscience and Nanotechnology*. McGraw-Hill Education (India), 2012.

Nagar, A. and Pradeep, T., Clean Water through Nanotechnology: Needs, Gaps, and Fulfillments, *ACS Nano* 2020, 14, 6420–6435.

T. Pradeep, *NANO: The Essentials: Understanding Nanoscience and Nanotechnology*, McGraw Hill Education, (2017).

<https://pradeepresearch.org/>

<https://iccw.world/>

<https://pradeepresearch.org/towards-an-arsenic-free-punjab-2/>

<https://pradeepresearch.org/news/general-highlights/>

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Prof. Pradeep's work is in the area of molecular materials and surfaces. His group discovered that noble metal nanoparticles degrade halocarbons efficiently to amorphous carbon and metal halides at room temperature and at low concentrations. This discovery has led to the world's first nanochemistry based water filter for pesticide removal as many pesticides of relevance are halocarbons. This technology is estimated to have reached about 9 million people. His group developed several technologies to remove other contaminants from drinking water. Combining several such materials, an all-inclusive affordable drinking water purifier has been developed. Exciting aspect of this technology is the creation of advanced materials by simple and environment-friendly methods. This technology, named AMRIT, is being implemented now in the arsenic affected regions of India. About one million people have been benefitted from these installations. Several other drinking water technologies have been rolled out from his lab. To take such technologies forward, four companies been incubated with the participation of IIT Madras. This activity is now being expanded globally.



# 9

## Azoreductase Activity of Azo Dye- Decolorizing Bacteria Isolated from Biocompost

Chem Chanchao, Ph.D. Student, Department of Environmental Engineering Science, Gunma University, Japan

### Highlight

- Newly isolated *B. subtilis* strain CKCC was isolated from biocompost
- *B. subtilis* strain CKCC decolorized azo dye (RR120) by azoreductase activity
- Azoreductase activity was recorded with 174.16 U/g under the optimum conditions
- Degradation of the chemical structure of RR120 was elucidated by FT-IR analysis



Figure 1: Biodecolorization efficiency analysis.

### I. Introduction

Azo dyes are categorized as one of the most widely utilized in the textile industry (Guadie *et al.*, 2017). The azo dyes are chemically represented as  $R_1-N=N-R_2$ , where  $(-N=N-)$  is the azo group, and the  $R_1$  or  $R_2$  can be either aryl or alkyl compounds (Chung, 2016). The textile industry generates an extensive number of effluents annually, resulting in significant dye pollution. This pollution poses a threat to ecosystems, displaying harmful effects like mutagenicity, genotoxicity, and carcinogenicity in both humans and animals (Telke *et al.*, 2015). Previous research had already confirmed that bacteria are able to degrade azo dye structures with azoreductase activities (Sarayu & Sandhya, 2010). Therefore, this study aimed to screen and isolate the newly potential azo dye decolorizing bacterium from biocompost.

## II. Materials and Methods

### 2.1 Screening and Isolation of Azo Dye-Decolorizing Bacteria

The screening and isolation were conducted in 50 mL of nutrient broth (pH 7.0), which was inoculated with (5%, w/v) biocompost and supplemented with azo dye Reactive red 120, (RR120) 50 mg/L and incubated at 37°C by 200 rpm for 48 hrs. The culture was collected and then centrifuged (Kubota 5922, Japan) at 8,000 × g for 5 minutes, and the resulting supernatants were analyzed using a UV-Vis spectrophotometer (Shimadzu Model UV-160, Japan) at the respective dye wavelengths ( $\lambda_{\text{max}} = 537 \text{ nm}$ ). The percentage of azo dye decolorization efficiency was calculated using equation (1). The potential RR120 removals were aseptic serial dilution in 0.85% NaCl and spread on nutrient agar plates, and incubated at 37°C for 24 hrs. Finally, the morphologically different single colonies were isolated.

$$\text{Decolorization efficiency (\%)} = \frac{\text{OD Initial} - \text{OD Degraded}}{\text{OD Degraded}} \times 100 \quad (1)$$

Where OD Initial refers to the initial absorbance, OD Degraded is the absorbance after dye decolorization.

### 2.2 Strain Identification and Azoreductase Activity

The pure colonies of the potential azo dye decolorization were isolated. The genomic DNA was extracted by using the DNeasy Blood & Tissue Kit. The 16S rRNA gene was amplified using PCR using the universal eubacteria-specific primers. The PCR procedure was performed with standard conditions. Each sequencing data was analyzed by BioEdit Sequence Alignment Editor Program and compared with the NCBI GenBank online database using BLASTn. Then, each isolated strain was prepared for azoreductase assay by using methyl red as the substrate, as described in a previous study (Qi *et al.*, 2017).

### 2.3 Fourier Transform-Infrared Spectroscopy (FT-IR) Analysis

Biodegradation residues were characterized by FT-IR. Decolorized functional groups of RR120 before and after decolorization were determined. Different functional groups produced bond absorptions at various locations and intensities on the IR spectrum. IR absorptions of common functional groups will be compared with the standard control sample.

## III. Results and Discussion

### 3.1 Strain Identification and Azo Dye Decolorization Efficiency

As a result, strain CKCC was considered in this study. The partial sequence of the 16S rRNA gene was 1,411 bp. After BLAST with GenBank online databases, the result revealed that the strain CKCC showed the highest similarity with *Bacillus subtilis* strain X-c in 99.35%. Therefore, the strain CKCC was classified as *B. subtilis* strain CKCC. The study results show that *B. subtilis* strain CKCC efficiently decolorizes RR120 by approximately 89.88% within 96 hrs under the optimum conditions by pH 7.0 at 37°C under 200 rpm (Figure 1).

In this study, the *B. subtilis* strain CKCC was identified as an azo dye-degrading bacterium that has evolved the ability to break down and metabolize azo dyes. This degradation process is carried out by an enzyme called azoreductase, which is produced by the isolated strain CKCC. Azo dyes contain R1-N=N-R2, which forms their characteristic color. The strain CKCC produces the azoreductase enzyme that can cleave this

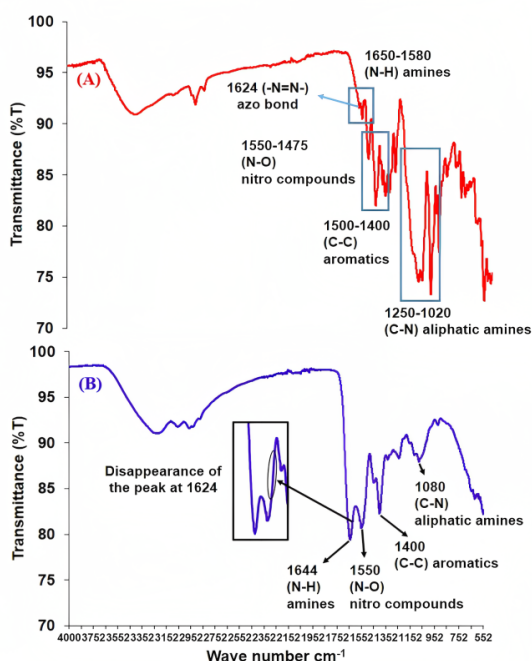
bond (-N=N-). The enzyme catalyzes the reduction of the azo bond, resulting in the formation of two aromatic amines. This process effectively breaks down the azo dye into smaller, less complex compounds (Ali *et al.*, 2022). The specific metabolic pathways may vary among different azo dye-degrading bacteria (Santhanarajan *et al.*, 2022).

The degradation of azo dyes by these specialized bacteria helps detoxify the dyes and reduces their impact on ecosystems. It's important to note that not all bacteria have the ability to degrade azo dyes. Azo dye-degrading bacteria have developed these capabilities as a result of natural selection, and their presence can be beneficial in environmental remediation efforts, such as the treatment of industrial wastewater containing azo dyes (Ali *et al.*, 2022). Recently, researchers have also been exploring the use of these bacteria in bioremediation and biotransformation processes to address azo-dye pollution.

### 3.2 Azoreductase Activity and FT-IR Analysis

The azoreductase activity was considered in this study from *B. subtilis* strain CKCC. As a result of azoreductase activities produced by strain CKCC was recorded with 174.16 U/g under the optimum conditions. Azoreductase catalyzes the reductive cleavage of azo bonds (-N=N-) to give colorless. The functional groups of azo dye (R1-N=N-R2) were broken down into an aryl group, aromatic amine, or arylamine as an aliphatic (Chung, 2016). In the presence of an electron donor (often a cofactor like NADH or NADPH), azoreductase catalyzes the transfer of electrons to the azo group, breaking the nitrogen-nitrogen double bond. This process transforms the azo group into two amino (-NH<sub>2</sub>) groups. In this reaction, R1 and R2 are organic moieties that make up the rest of the azo dye molecule. The products of this reduction reaction are two aromatic amines. These aromatic amines are typically less complex and less colorful than the original azo-dye molecules (Kapoor *et al.*, 2021). Furthermore, azo dye-degrading bacteria can further metabolize these aromatic amines, utilizing those molecules for energy into their biomass (Mittal *et al.*, 2022). Moreover, the FT-IR analysis showed evidence of the disappearance of the peak at 1624 cm<sup>-1</sup> assigned to (-N=N-) of azo groups (Figure 2).

Figure 2. The FT-IR analysis of azo dye (RR120), (A) control of decolorization by RR120, (B) metabolic residues of RR120 after decolorization in 96.0 hrs.



The absence of (-N=N-) in FT-IR spectra culture presents that RR120 completely catalyzes the reductive cleavage of azo bonds. Based on the FT-IR results, it can be clearly concluded that the RR120 was successfully degraded by the azoreductase produced by the *B. subtilis* strain CKCC. The perspective pathway of RR120 using *B. subtilis* strain CKCC was elucidated in Figure 3.

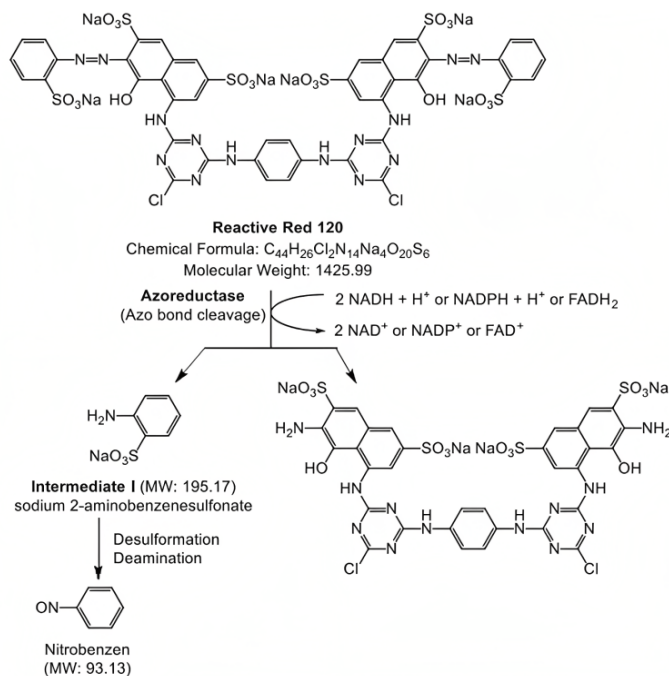


Figure 3. The perspective pathway of RR120 by using *B. subtilis* strain CKCC. Adapted from Shahi *et al.*, (2021).

#### IV. Conclusion

The potential azo dye decolorizer *B. subtilis* strain CKCC exhibited extracellular azoreductase activity. The FT-IR analysis of the degradation products illustrated that decolorization was attained via biodegradation of dye by-products of immobilized *B. subtilis* strain CKCC. Therefore, the *B. subtilis* strain CKCC was considered an excellent candidate for textile effluent treatment.

#### Reference:

- Ali, S. S., Al-Tohamy, R., Mahmoud, Y. A. G., Kornaros, M., Sun, S., & Sun, J. (2022). Recent advances in the life cycle assessment of biodiesel production linked to azo dye degradation using yeast symbionts of termite guts: A critical review. *Energy Reports*, 8, 7557-7581. <https://doi.org/10.1016/j.egy.2022.05.240>.
- Chung, K. T. (2016). Azo dyes and human health: A review. *Journal of Environmental Science and Health, Part C*, 34(4), 233-261. <https://doi.org/10.1080/10590501.2016.1236602>.
- Guadie, A., Tizazu, S., Melese, M., Guo, W., Ngo, H. H., & Xia, S. (2017). Biodecolorization of textile azo dye using *Bacillus* sp. strain CH12 isolated from alkaline lake. *Biotechnology reports*, 15, 92-100. <https://doi.org/10.1016/j.btre.2017.06.007>.
- Kapoor, R. T., Danish, M., Singh, R. S., Rafatullah, M., & HPS, A. K. (2021). Exploiting microbial biomass in treating azo dyes contaminated wastewater: Mechanism of degradation and factors affecting microbial efficiency. *Journal of Water Process Engineering*, 43, 102255. <https://doi.org/10.1016/j.jwpe.2021.102255>.

- Mittal, Y., Dash, S., Srivastava, P., Mishra, P. M., Aminabhavi, T. M., & Yadav, A. K. (2022). Azo dye containing wastewater treatment in earthen membrane based unplanted two chambered constructed wetlands-microbial fuel cells: A new design for enhanced performance. *Chemical Engineering Journal*, 427, 131856. <https://doi.org/10.1016/j.cej.2021.131856>.
- Qi, J., Anke, M. K., Szymańska, K., & Tischler, D. (2017). Immobilization of *Rhodococcus opacus* 1CP azoreductase to obtain azo dye degrading biocatalysts operative at acidic pH. *International Biodeterioration & Biodegradation*, 118, 89-94. <https://doi.org/10.1016/j.ibiod.2017.01.027>.
- Santhanarajan, A. E., Rhee, C., Sul, W. J., Yoo, K., Seong, H. J., Kim, H. G., & Koh, S. C. (2022). Transcriptomic Analysis of Degradative Pathways for Azo Dye Acid Blue 113 in *Sphingomonas melonis* B-2 from the Dye Wastewater Treatment Process. *Microorganisms*, 10(2), 438. <https://doi.org/10.3390/microorganisms10020438>.
- Sarayu, K., & Sandhya, S. (2010). Aerobic biodegradation pathway for Remazol Orange by *Pseudomonas aeruginosa*. *Applied biochemistry and biotechnology*, 160, 1241-1253. <https://doi.org/10.1007/s12010-009-8592-1>.
- Shahi, A., Chellam, P. V., Singh, R. S., & Verma, A. (2021). Biodegradation of reactive red 120 in microbial fuel cell by *Staphylococcus equorum* RAP2: statistical modelling and process optimization. *Journal of Water Process Engineering*, 40, 101913. <https://doi.org/10.1016/j.jwpe.2020.101913>.
- Telke, A.A., Kadam, A.A., Govindwar, S.P. (2015). Bacterial Enzymes and Their Role in Decolorization of Azo Dyes. In: Singh, S. (eds) *Microbial Degradation of Synthetic Dyes in Wastewaters*. *Environmental Science and Engineering*. Springer, Cham. [https://doi.org/10.1007/978-3-319-10942-8\\_7](https://doi.org/10.1007/978-3-319-10942-8_7).



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# Intelligent Transport System (ITS) for Traffic Management in Phnom Penh City

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Tauch Sotharith, Master's Degree, Master of Computing, New Zealand

## Highlight

- The key functionalities of ITS that could benefit traffic management in Phnom Penh city
- Review of the existing traffic management systems in Phnom Penh City
- Future IST systems for Traffic management in Phnom Penh city

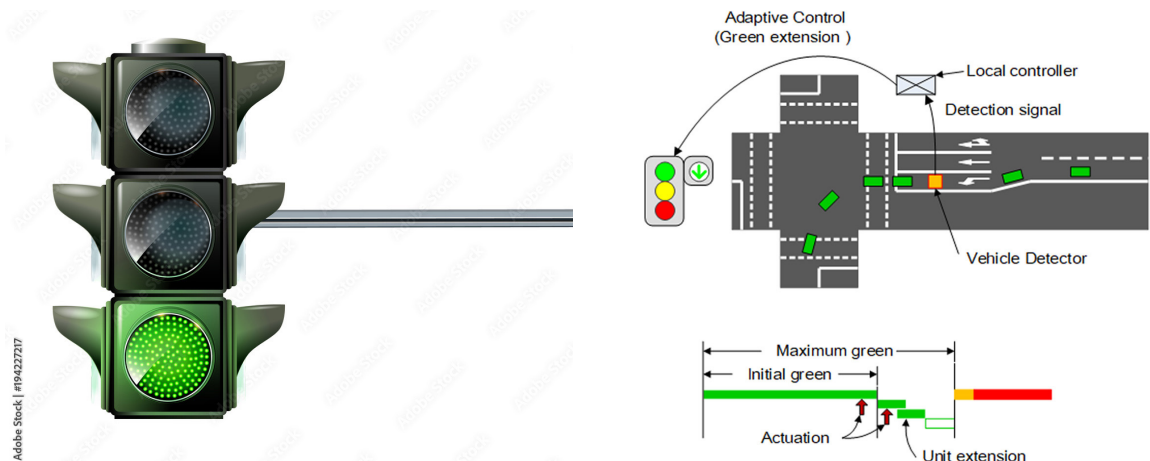


Figure 1. Green Traffic light and adaptive control applied to left turn phase

## I. Introduction

Traffic congestion has become an increasing concern in Phnom Penh City, the capital of Cambodia, due to the increasing demand for personal transport and the lack of public transport. According to the Ministry of Public Work and Transport report, the total number of vehicles registered from year 1990 to year 2022 is 6,877,269. This includes 5,844,236 motorcycles, 744,755 small vehicles, and 288,278 trucks (MPWT, 2023). The root causes of traffic congestion and traffic accidents are human factors, vehicle factors, and road conditions. This issue has become a major concern and challenge for the Government as well as the Land Transport Authority of Cambodia. Road widening can be considered as one option; However, to directly tackle the problem of traffic congestion and traffic accidents, the Intelligent Transport System (ITS) is considered a vital solution for traffic management, which has been adopted in many developed countries.

ITS is an advanced technological innovation that can be utilized as a tool designed to improve the effectiveness, efficiency, and safety of the transport sector across the globe (Divya, Gurdit, and Sanjeev, 2013). ITS contains several components and functionality that can be implemented to suit specific requirements. More details will be described in section III.

## II. Review of the Existing Traffic Management Systems in Phnom Penh City

Phnom Penh is the capital city and the most populated area of Cambodia. The traffic conditions in the city have become a concern topic. According to the Phnom Penh City Urban Transport Master Plan, studied by the Japan International Cooperation Agency (JICA) in 2001, the transport issues in the Phnom Penh Metropolitan Area need to be addressed. This master plan predicted that the problem would become a major social issue in the future (Katahira & Engineering International, 2001). Recently, this master plan was revised by JICA to update the current situation of urban transport in Phnom Penh and then propose solutions to the identified issues.

JICA conducted a data collection survey in Phnom Penh City and identified most traffic facilities in Phnom Penh are outdated and lack maintenance. In 2014, JICA proposed a project called “The Project for Comprehensive Urban Transport Plan in Phnom Penh Capital City”. In this project, two Action plans were proposed (1) introduction of City Bus System and (2) Comprehensive Traffic Management Plan in the City Center. This paper focuses solely on Traffic Management, thus, public transport will not be included.

The survey conducted by JICA in 2014 found that most traffic facilities in Phnom Penh are outdated and recommended short-term and medium-term plans for the signalized intersections across the city. Following the request of the government of Cambodia, the Japanese Government provided “Grant Aid” to Phnom Penh Capital Administration (PPCA) through the Japan International Cooperation Agency by integrating

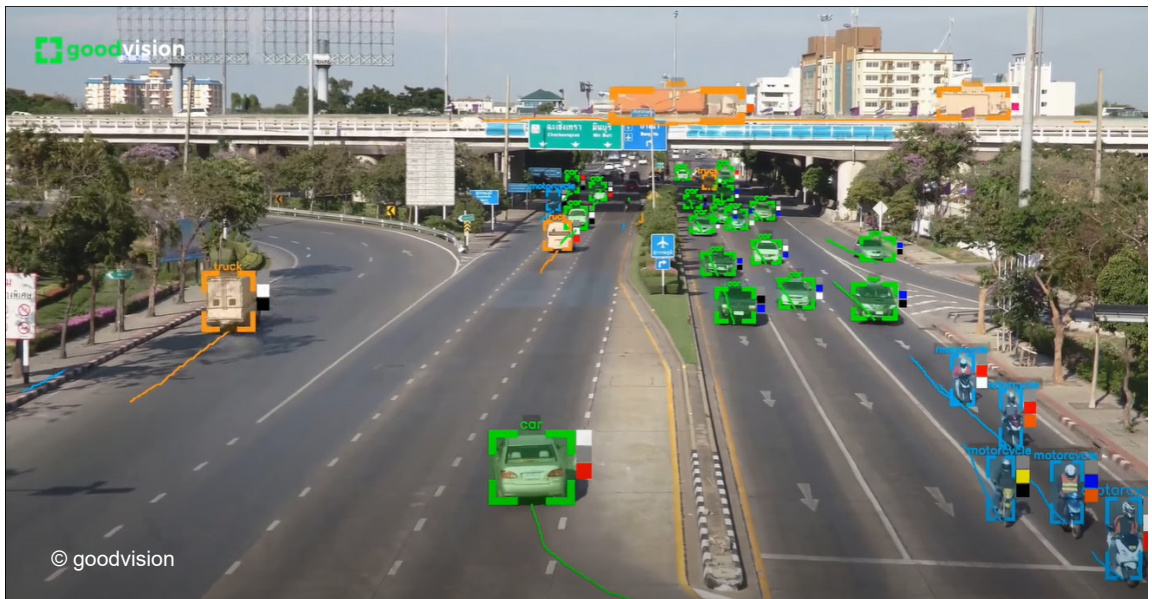


Figure 2. ATMS monitors the real-time traffic flow

traffic signals at 69 intersections across Phnom Penh city for the short-term plan (JICA, 2023). According to Matsuoka in 2018, the medium-term plan was implemented by connecting all previously installed traffic signals to the Area Traffic Control System. The entire system contains 109 traffic signals, 196 vehicle detectors, and 26 traffic surveillance video cameras. These devices can record and send traffic information to the Traffic Control Center at the PPCA.

Currently, three types of ITS components have been introduced and implemented in Cambodia. The Area Traffic Control System, the Smart Traffic Management systems, the air control quality are implemented in Phnom Penh city by PPCA and MPWT. AI-Weight In Motion and the Electric Toll Collection (ETC) are deployed in Kandal province and the new expressway Phnom Penh-Sihanoukville by MPWT and China Road

and Bridge Corporation (CRBC). However, these components are implemented in isolation, which in this case each component are not controlled by a single Government entity.

### III. ITS Functionalities for Traffic Management in the Urban City

#### Smart Traffic Light

Generally, the traffic signal is used to control the flow of traffic at critical locations such as major intersections, in front of the emergency services department, and at railway crossings. The Traffic Control System controls the duration of red light and green lights in real-time based on the flow volume of vehicles along the road toward an intersection. In this case, the duration of the green light will be extended based on the direction of the largest volume of vehicles (Ghazal et al, 2016). With the help of AI cameras and detectors, traffic data are collected and analyzed by the system at the control center, and then disseminate traffic information to road users in real-time. The study by Aston University found that using AI traffic lights can reduce traffic congestion and keep traffic flow by reading live camera footage and adjusting traffic lights accordingly (Aston University, 2023). The following Figure 1 shows an example of the Smart Traffic Light (Adobe Stock & Matsuoka, 2018 ).

#### Advanced Traffic Management System (ATMS)

ATMS is a system that manages, controls, and monitors traffic flow in real-time on any designated road network. The system is equipped with various sensors, cameras, communication devices, and data processing technologies to collect, and analyze traffic information, and then take actions to improve the flow of traffic and safety. Figure 2 shows an example of the ATMS (GREENIP).

#### Vehicle to Vehicle (V2V) Technology

V2V is a technology that enables vehicles to exchange information about speed, location, heading wirelessly, and other parameters. This technology supports road safety, traffic efficiency, and driver experience by

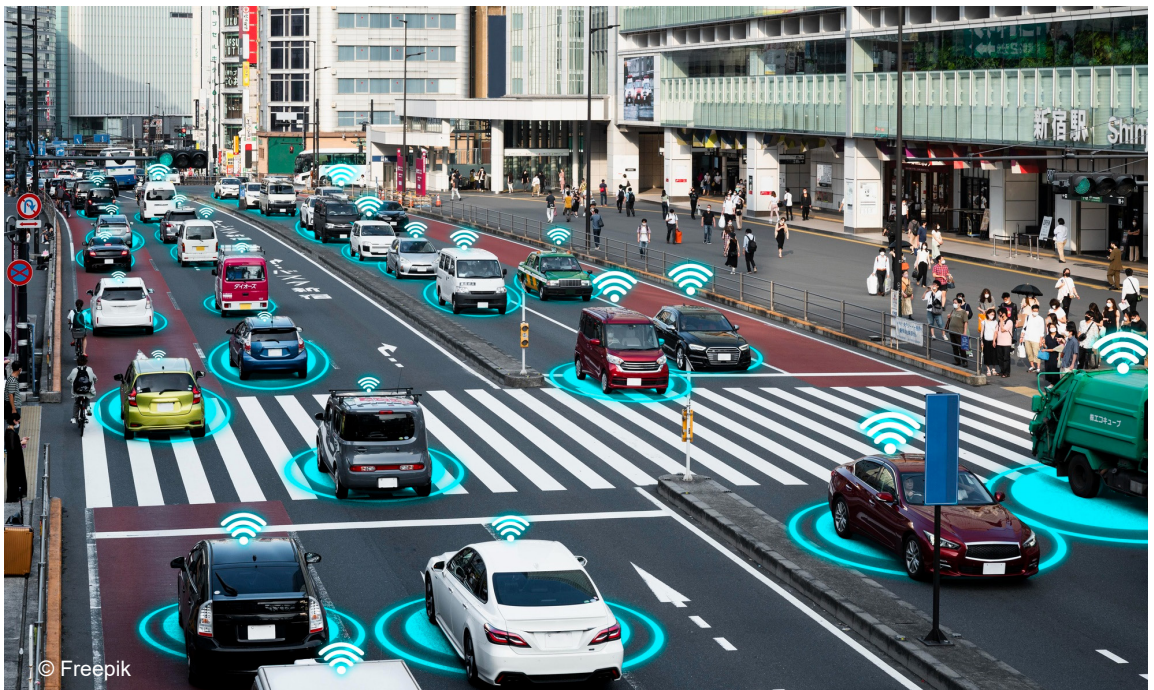


Figure 3: Interconnection between vehicles using V2V



providing alerts or warnings of hazards, collisions, or delays that are happening ahead. All vehicles within a range of 300 meters can communicate seamlessly with one another. They can interact with traffic signs, road signs, or rail/bus crossings (U.S. Department of Transport, 2014). Figure 3 shows a representation of the V2V (eInfochips).

### Cooperative-ITS (C-ITS)

C-ITS is a part of the Intelligent Transport Systems (ITS) that capable of enhancing ITS communication between vehicle to vehicle, vehicle to infrastructure, and so on. This technology provides reliable information relating to road conditions such as traffic volume, potential hazards, and collisions to road users for safe decision-making while on the road (ISO and CEN, 2020). Furthermore, effective data exchange can be done through wireless technologies that allow communication between vehicle to vehicle and vehicles to infrastructure. C-ITS supports various specialized applications such as cooperative adaptive cruise control, collision warning and avoidance, platooning, lane change assistance, intersection management, and emergency vehicle priority. Figure 4 shows the C-ITS broadcasting accident information via wireless connectivity.

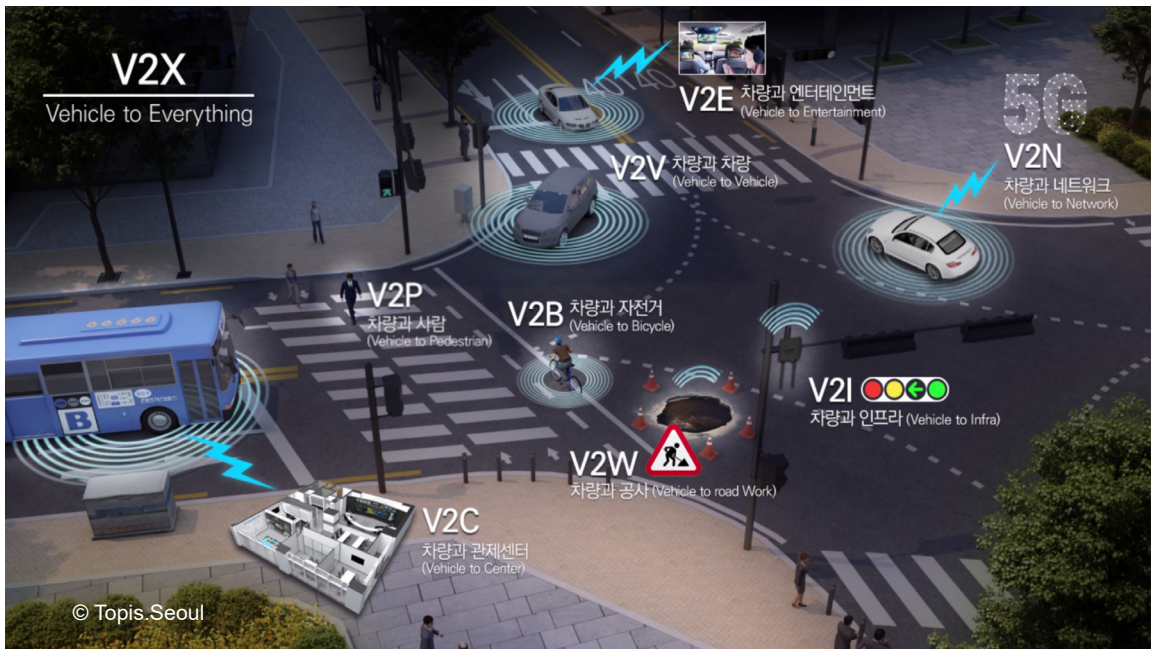


Figure 4: C-ITS wireless connectivity between vehicles, pedestrians, and infrastructure

### Traffic Data/Management Center:

The Traffic Data/Management Center is the core component of the traffic management system. ITS has various components and functions. Without a data center, every component is operated in isolation. To effectively manage all ITS components as one system, all the components need to be integrated and controlled at a Traffic Data Center. In this case, data and information are effectively stored, managed, and distributed traffic conditions to road users more efficiently. With the operation team, the traffic management center can respond on time to any traffic matter on the street (Tech Xplore, 2022).



Figure 5: Traffic Data Center, Land Transport Authority in Singapore

## VI. Conclusion

The adoption of ITS helps improve traffic management tasks such as reducing congestion, reporting accidents, reducing travel time, and cost, and enhancing mobility, accessibility, comfort, and convenience for all road users. Furthermore, sustainable traffic management can be fully dependent on ITS technology for the growth of existing cities or new city development in the future. Traffic management is a complicated issue and task that requires stakeholders within Government entities and the private sectors to collaborate in order to achieve a better solution. New technologies such as Smart Traffic Light, Advanced Traffic Management System (ATMS), Vehicle to Vehicle (V2V) Technology, and Cooperative-ITS (C-ITS) are the key potential solutions to the traffic problem that Phnom Penh City is currently facing. ITS is a technology that has been utilized in the Smart City, which makes a city become a liveable city.

## Reference:

- Aston University (2023). Accessed September 9, 2023. AI traffic light system could make traffic jams a distant memory. <https://www.aston.ac.uk/latest-news/ai-traffic-light-system-could-make-traffic-jams-distant-memory-0>
- B. Ghazal, K. ElKhatib, K. Chahine and M. Kherfan, "Smart traffic light control system," 2016 Third International Conference on Electrical, Electronics, Computer Engineering and their Applications (EECEA), Beirut, Lebanon, 2016, pp. 140-145.
- Constant Tech (2022). Accessed September 09, 2023. Traffic Management Centers. Constant Technologies, INC. <https://constanttech.com/traffic-management-centers>.
- Divya. B, Gurdit. S and Sanjeev. S. (2013). Intelligent Transportation System for Developing Countries A Survey. International Journal of Computer Applications, 33.
- General Department of Land Transport (2023). Accessed September 10, 2023. Truck Modernization Strategic Plan 2022-2023. <https://www.mpwt.gov.kh/kh/documents/policy/557/>
- ISO & CEN (2020). Accessed September 9, 2023. Cooperative Intelligent Transport Systems (C-ITS) Guidelines on the usage of standards. Technical Reports TR 21186. <https://www.itsstandards.eu/app/uploads/sites/14/2020/10/C-ITS-Brochure-2020-FINAL.pdf>
- JICA (2023). Accessed September 9, 2023. Data Collection Survey on Urban Transport in Phnom Penh – Final Report. [https://openjicareport.jica.go.jp/pdf/12371746\\_01.pdf](https://openjicareport.jica.go.jp/pdf/12371746_01.pdf)
- Katahira & Engineering International (2001). Accessed September 9, 2023. The study on the transport master plan of the Phnom Penh Metropolitan Area in the Kingdom of Cambodia. [https://openjicareport.jica.go.jp/pdf/11674850\\_01.pdf](https://openjicareport.jica.go.jp/pdf/11674850_01.pdf)
- Matsuoka. S (2018). Traffic management project in Phnom Penh. IATSS Research, 42(4), 180-189.
- MPWT (2023). Annual Report for Year 2022 and Action Plan for Year 2023 for the Ministry of Public Works and Transport. General Department of Planning and Policy, Ministry of Public Works and Transport.
- Tech Xplore (2022). Accessed September 9, 2023. AI traffic light system could make traffic jams a distant memory. <https://techxplore.com/news/2022-05-ai-traffic-distant-memory.html>
- U.S Department of Transport (2014). Accessed September 9, 2023. Vehicle-to-Vehicle Communication Technology. [https://www.nhtsa.gov/sites/nhtsa.gov/files/documents/v2v\\_fact\\_sheet\\_101414\\_v2a.pdf](https://www.nhtsa.gov/sites/nhtsa.gov/files/documents/v2v_fact_sheet_101414_v2a.pdf)



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