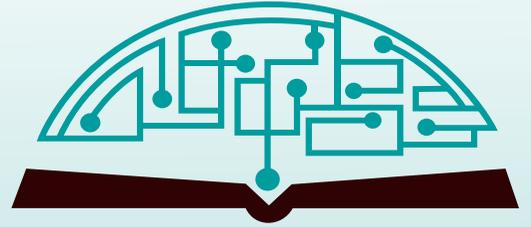


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Significant Mutations of Hantaviruses Contribute to Its Cytotoxicity

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ABSTRACT: Hantaviruses are pathogens that belong to the *Bunyaviridae* family which are segmented negative-strand RNA viruses. Just as many other negatively stranded RNA viruses, its RNA-dependent RNA polymerase (RdRp), also referred to as L protein of hantaviruses, lacks capping activity, which enhances mRNA processing, mRNA export, and translation. Hantaviruses, on the other hand, employ the use of “cap snatching”, in which 5' capped oligonucleotides are cleaved from infected cell transcripts and used as primers to initiate the viral mRNA synthesis and it is known that the cytotoxicity of a hantavirus depends on the effectiveness of its cap-snatching domain. In this article, the correlation between the viral toxicity and its cap-snatching domain (first 220 amino acids of the L protein) is explored. As it has been found by aligning the RdRp residue cap-snatching domains, certain mutations can completely reduce the viral cytotoxicity.

KEYWORDS: Computational Biology and Bioinformatics; Computational Biomodeling; Sequence alignment; Cap-snatching domain.

Introduction

Hantaviruses belong to the *Bunyaviridae* family, which is a group of segmented negative-strand RNA viruses that include ones which cause severe human diseases.¹⁻³ Hantaviruses get significant attention as emerging pathogens which are expanding their global distribution.⁴⁻⁷ In Asia, the prototypic Hantaan virus is a causative agent for hemorrhagic fever with the renal syndrome with fatality rates of about 3%. In South and North Americas, the hantaviruses Sin Nombre and Andes are associated with hantavirus cardiopulmonary syndrome with up to 40% mortality.⁷⁻¹² There is currently no licensed vaccine against hantaviruses and therapeutic options are limited.¹³

Just as many other RNA viruses, hantavirus RNA dependent RNA polymerase uses a unique “cap snatching” mechanism for transcription initiation, as viral mRNAs are around 100 nucleotides shorter than parent genomic viral RNAs, and lack a poly-A tail.¹⁻³ During the cap-snatching process, 10-14 nucleotides' long 5'-capped oligonucleotides are cleaved from host cell transcripts and used as primers to initiate the viral mRNA synthesis, following a prime-and-realign mechanism. Viral mRNAs are translated into the cell cytoplasm by the host cell translation machinery.⁴⁻⁷ (Figure 1)

Viral RdRp also replicates the genome through the process of complementary cRNA synthesis. Complementary cRNAs are the precise complement of genomic viral RNA (vRNA) and performance templates for the synthesis of the negative-sense viral genome. Viral assembly and maturation occur on the cell surface or on the Golgi body. Virions that mature on the Golgi body are transported to the cell surface through the vesicular secretory pathways located in the cytoplasm. As a result, new virions bud off the host cells from the cytomembrane.¹⁻³

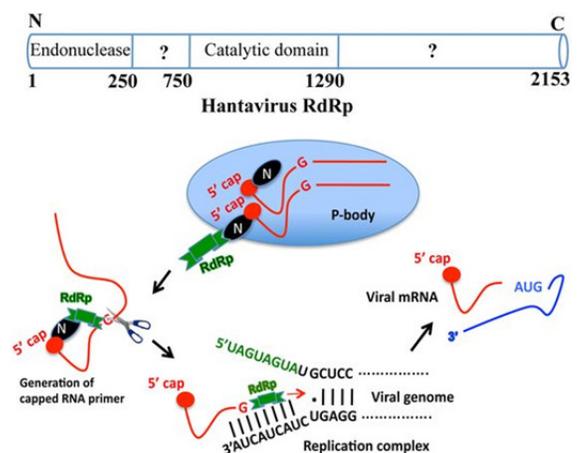


Figure 1: The cap-snatching mechanism. The diagram illustrates the cap-snatching mechanism of the RdRp and how it is involved in the viral replication in the host cell.¹⁴

RNA-dependent RNA-polymerase:

Hantavirus RdRp (RNA dependent RNA polymerase) is a large protein with a mass of 250-280 kDa. RdRp mediates both transcription and replication of the viral genome. During transcription, RdRp synthesizes viral mRNA from a negative-sense vRNA template.¹⁻³ During replication, RdRp replicates the viral RNA genome via a cRNA intermediate. Thus, it is likely that hantavirus RdRp has multiple activities, including endonuclease (cap-snatching activity), replicase, transcriptase, and RNA helix unwinding activities.¹³

Due to their essential role in virus multiplication, the conserved endonucleases of RdRp of segmented negative-strand RNA virus polymerases are of great interest to basic virus research. Their enzymatic activity makes them attractive drug

targets for therapeutic intervention. Here, previous studies were confirmed and extended, providing further evidence for the importance of the cap-snatching domain for viral toxicity.¹³

Importance and Hypothesis:

In the infected host cell, cap-snatching is a mechanism of great interest as it is responsible for the initiation of virus replication. Consequently, the interruption in cap-snatching through certain mutations in the viral RdRp will inhibit virus replication and will likely improve the prognosis of hantavirus disease. Our studies present the mutations of the cap-snatching domain of five different hantaviruses, as it was believed that certain mutations would completely reduce the viruses' cytotoxicity or make them even more dangerous. The study was executed to give a deeper insight into the RdRp endonuclease domain and its mutations. Identification of certain mutations will potentially reveal a novel therapeutic target for combating this viral illness.

■ Methods

Sequence alignment:

The sequence alignment was executed by web platforms such as NCBI Protein Blast (National Institute of Health, Maryland, US) and Clustal W (EMBL-EBI, Cambridgeshire, UK).²⁶ The sequences of Andes and Maporal virus were compared to Hantaan virus endonuclease (access ID is 5ize at Protein Data Bank (PDB) RCSB, US). The first 220 amino acids in the sequence were taken out to the whole L protein sequence, to focus on the cap-snatching domain only. Only then they were aligned and checked for mutations. The reference codes used for L Protein Cap-snatching domains of hantaviruses are as follows: Puumala virus (GenBank: BAJ14125.1), Hantaan virus (GenBank: AAG10042.1), Maporal virus (NCBI Reference Sequence: YP_009362282.1), Tula virus (NCBI Reference Sequence: NP_942124.1), Andes virus (GenBank: AAG22533.4).

Swiss-Model:

The Andes hantaviruses (PDB: 5hsb) and Hantaan virus (PDB: 5ize1.A) templates for MAPV modeling were selected in SWISS-MODEL (Swiss Institute of Bioinformatics, Basel, Switzerland) template library supported Protein Data Bank (PDB) by NCBI.¹⁴ The protein sequences were copied and aligned with the corresponding template sequences. The variable regions which include insertions and deletions were rebuilt by the fragment library. The side chains were added later. Consistent with land Protocols, the geometry of the model was maintained by a physical phenomenon. The model also can be built by using PROMOD-II. The standard of the model was estimated by QMEAN. A score closer to 0 demonstrates the best quality. The scores relatively closer -1 were not used. QMEAN consists of four components: (i) the neighboring C β atoms' interaction value, (ii) all atoms interaction, (iii) the solvation potential, (iv) the torsion potential. All scores were recorded. The similarity of organic compound alignment was estimated by the 'local quality estimate' analysis executed by the server. The values smaller than 0.6 were avoided and only the upper values were accepted. Consequently, the model was tested for the standard by comparing

avoided and only the upper values were accepted. Consequently, the model was tested for the standard by comparing its QMEAN mean score to other rebuilt models. The model was illustrated with its variance. Importantly, the model was accepted if it is within the range of mean giving only one unit of ordinary deviation.

The model of hantaviruses comes with manganese atoms; therefore, it is important to pick the high-quality ligand alignment. The ligand modeling was selected by using four criteria based on the template alignment of the model: (i) the biologically relevant ligands, (ii) the ligand contact with the model, (iii) the absence of ligand clashing, (iv) the conserved sequences alignment where they interact with ligands. The loss of one of the standards accustomed assumes that the model does not satisfy the analysis. At the same time, the accuracy of the tertiary structure was estimated by the GMQE score, which ranges from 0 to 1. The upper score shows the upper reliability of the structure.

Theoretical pI/Mw:

The calculation of theoretical pI/Mw of each N terminus cap-snatching domain was executed by ExPASy pI/Mw computing tool. The first 220 amino acids in the sequence of each chosen hantavirus were applied. Protein pI is calculated using pK values of amino acids, which were defined by examining polypeptide migration between pH 4.5 to 7.3 in an immobilized pH gradient gel environment with 9.2 M and 9.8 M urea at 15 °C or 25 °C. Protein Mw was calculated by the addition of average isotopic masses of amino acids in the protein and the average isotopic mass of one water molecule. Molecular weight values were given in Dalton (Da).

■ Results and Discussion

Previous studies have shown the existence and the functionality of an endonuclease in the N-terminal domain of the hantavirus genus. This research sought to demonstrate certain mutations in the cap-snatching domain of the 5 hantaviruses. It has shown that there are certain amino acids, the mutations of which, will completely decrease the toxicity of the virus and consequently its lethality.

First, the N terminus (first 220 amino acids of the L protein) of five hantaviruses were aligned, showing the evolutionary distances between them (Figure 2). This shows how *Tula* and *Puumala* viruses are closely related. They are 85.91 % identical (Table 1), which would explain their similar cytotoxicity with mortality rates of less than 1 %.¹⁵⁻¹⁸ At the same time, it was observed that about 13 % of the protein sequence differentiates *Puumala* and *Tula* viruses from a toxic *Andes* virus which has a mortality rate of between 40 % and 50 % in South America.¹⁹ As a result, it can be assumed that this 13 % of the protein cap-snatching domain significantly changes the cytotoxicity of the virus. This shows that by looking at those mutations that are present in one virus and do not occur in the other one, which mutations actually do affect the toxicity of the hantavirus can be determined.

A detailed alignment of five hantaviruses was executed, allowing exact mutations and differences between the highly toxic and relatively safe viruses to be seen (Figure 3). To illu-

minate insignificant mutations that are very unlikely to change the functionality of the cap-snatching domain, the mutations in which the amino acid was replaced by the one with a similar functional group and properties were ignored. For instance, the tenth amino acid in the sequence is K in *Hantaan* but R in the other 4 hantaviruses, however, they both are positively charged, thus their functionality is very similar, and it should not strongly affect the functionality of the virus.

Table 1: The genetic distance difference in the percentage value of five hantaviruses. The table was built by the comparison of the L protein cap-snatching domain performed by the Clustal Omega web platform (version by 23 August 2020).

	Hantaan	Andes	Maporal	Puumala	Tula
Hantaan	100.00	71.82	70.45	67.73	68.64
Andes		100.00	88.18	76.82	77.27
Maporal			100.00	76.82	78.64
Puumala				100.00	85.91
Tula					100.00

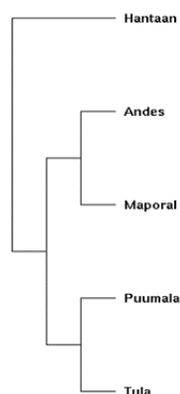


Figure 2: Phylogenetic tree of five *Hantaviridae* genus species and their genetic difference distance. The diagram shows the phylogenetic tree between five hantaviruses illustrating the common ancestors based on the L protein alignment. The cladogram was built by the Clustal Omega web platform (version by 23 August 2020).



Figure 3: Alignment of the 220 amino acids of the L protein cap-snatching domains. The figure illustrates the alignment of the active centers of 5 Hantaviruses. The red color stands for a hydrophobic residue; green - polar and blue - negative with pink - positive. The numbers on the right side of the row tell the number of amino acids in the row, performed by the Clustal Omega web platform (version by 23 August 2020).

Next, the active sites of each of the endonuclease domains with the most conserved amino acids were defined (Figure 4). None of the mutations in the conserved amino acids were found. Therefore, it can be concluded that the observation supports previous studies which imply that one of the mutations in the conserved amino acids would fully decrease the endonuclease activity of the protein, as these amino acids (H 36, E 54, D 97, E 110, T 112) are bonded with manganese atoms ligands.^{20,21,13} This helps to better understand the nature of the mutations and their specific location in the sequence which can be linked to its significance in the efficiency of the cap-snatching domain.²²



Figure 8: The protein sequence of the L Protein cap-snatching domain of five hantaviruses (220 amino acids). The diagram shows the amino sequence of the cap-snatching domains (first 220 amino acids). The connotation of each sequence above shows the isoelectric point and molecular weight of the protein sequence with the overall number of charged and polarized amino acids in the residue. This allows us to better understand the nature of the protein N terminus executed by the ExPASy - Compute pI/Mw tool.

Subsequently, the cap-snatching domains of both *Andes* and *Hantaan* viruses were aligned in 3D, as *Andes's* N terminus domain is evolutionarily the closest to *Hantaan's* domain. Moreover, both of them have a mortality of HNDV 10 – 12 % and ANVD 30 %. The most functionally important mutations are shown in the diagram (Figure 5). The 3D alignment reveals that the activities of both hantaviruses are similar and considering their relatively high cytotoxic activity it can be implied that the mutations on the cap-snatching domains do indeed affect its cytotoxicity.

Andes virus is similar to the *Maporal* virus by 88.18 %, being the closest related species in the *Hantaviridae* genus out of the five which have been chosen for this study. However, the mortality of the *Maporal* virus is about 1 %.²³⁻²⁶ All of the important mutations in the cap-snatching domain are shown Figure 6. It can be concluded that each of these mutations may decrease the toxicity of the *Andes* virus.

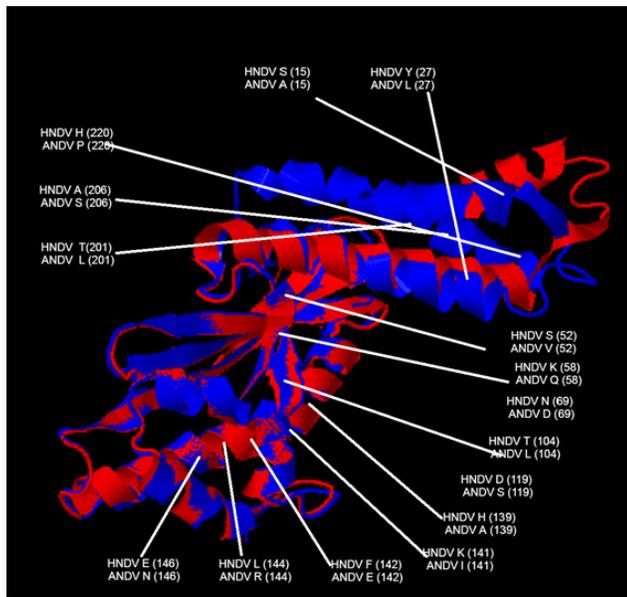


Figure 5: The 3D alignment of the *Hantaan* virus with *Andes* Virus. The diagram shows the important mutations on the aligned 3D models of the *Andes* virus and *Hantaan* virus L protein cap-snatching domain. The figure illustrates that the active site of the protein in both viruses is relatively similar.

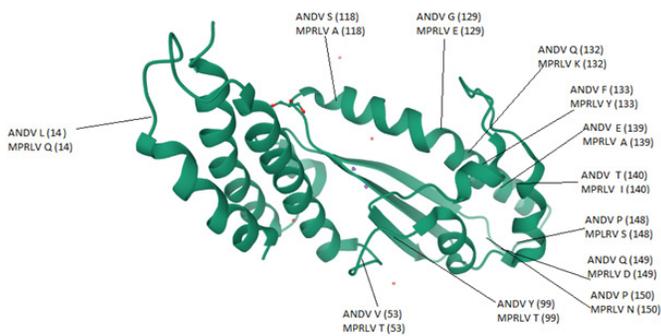


Figure 6: The comparison of the *Maporal* virus mutations on the *Andes* virus cap-snatching domain. The mutations of the *Maporal* hantavirus are displayed on the *Andes* virus cap-snatching domain. Each of these mutations significantly decreases viral toxicity.

Moreover, the *Andes* virus N Terminus model was used as a reference for *Tula* and *Puumala* viruses to demonstrate the important mutations with each of these viruses. Each of the demonstrated mutations on the *Andes* hantavirus cap-snatching domain reduces the cytotoxicity of the virus. This has revealed some common mutations such as ANDV E (141) and TULA H (141) with PUUM I (141).

This conclusion was revealed by executing a similar operation with *Tula* and *Puumala* virus. *Tula* and *Andes* viruses cap-snatching domains are similar with 77.27 % homology. The important mutations are shown below in Figure 7.

Andes and *Puumala* are similar with 76.82 % homology. Then their 3D models were also aligned, demonstrating the important mutations which result in such a big difference in cytotoxicity and as a result of mortality (Figure 8).

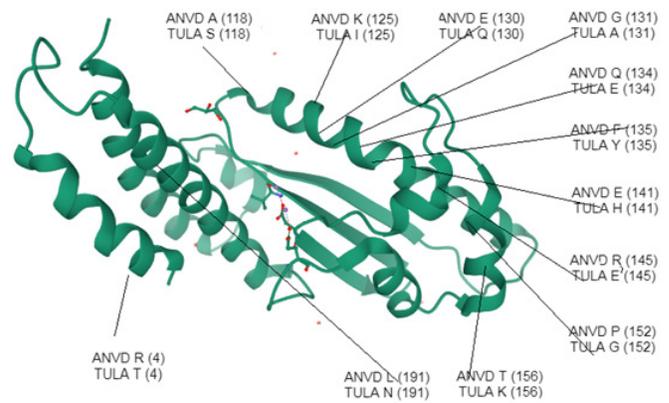


Figure 7: The comparison of the *Tula* virus mutations on the *Andes* virus 3D model. The Protein Data Bank Fasta file for the ANDV L Protein cap-snatching domain was used as a reference model to build the *TULA* virus cap-snatching domain. The model represents the mutations by comparing the number of the amino residue and the hantavirus revealing the mutations which decrease the toxicity of the virus.

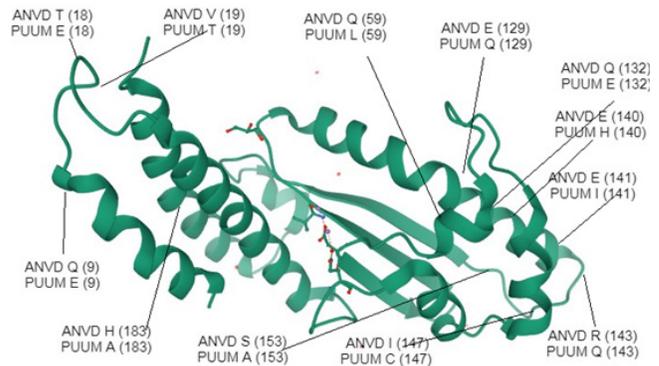


Figure 8: The comparison of the *Puumala* virus mutations on the *Andes* virus 3D model. The Protein Data Bank Fasta file for the ANDV L Protein cap-snatching domain was used as a reference model to build the *PUUM* virus cap-snatching domain. The model represents the mutations that appeared in the *PUUM* domain confirmation.

Conclusions

Hantaviruses are viruses that belong to the *Bunyaviridae* family which are segmented negative-strand RNA viruses. This means that its RNA-dependent RNA polymerase (RdRp) lacks capping activity and instead it makes use of a "cap-snatching" mechanism in which 5' capped oligonucleotides are cleaved from infected cell transcripts and used as primers to initiate the viral mRNA synthesis and it is known that the cytotoxicity of a hantavirus depends on its effectiveness of cap-snatching domain. According to our hypothesis, there may be certain mutations in the cap-snatching domain of the hantaviruses which would completely reduce the cytotoxicity of the virus or make it hypertoxic. Our experiment has aligned the viruses' endonuclease domains and demonstrated the most significant mutations in the amino sequences. By comparing the toxicity of the virus, which is linked to its mortality, it could be concluded that these mutations either reduce or increase their cytotoxicity, which proves our hypothesis.

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Ketsueki-Gata Through American Eyes: Examining the Correlation Between Blood Type and Personality

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ABSTRACT: In Japan, "Ketsueki-gata" has been a belief for over 90 years. It states that a person's ABO blood group significantly contributes to their demeanor. This is comparable to astrology, but blood type is a genetic component of humans and personality may also be influenced by genes. As the 1990 Minnesota Twin Study showed, twins that were raised apart developed similar social attitudes although they grew up in different environments, showing a correlation between genes and personality. This study tests if blood type influences personality and seeks to establish credibility of the personality-blood type phenomenon. Through an online survey, participants were asked to provide their blood type and complete a personality test that measured levels of openness to experience, conscientiousness, extroversion, agreeableness, and neuroticism. Results were converted into scaled scores, averaged, graphed, and analyzed using CHI square. Through the statistical analysis of 100 adults, their personality tests using the Big 5 Personality Traits model, and their blood type, no correlation was found. The null hypothesis of the CHI Square test failed to be rejected leading to the conclusion that blood type does not influence personality.

KEYWORDS: Behavioral and Social Sciences; Physiological Psychology; Blood-Type Personality Correlation; Big 5 Personality Traits; Ketsueki-Gata.

■ Introduction

A potentially pseudo-scientific belief that blood type influences personality began in 1927 with the publication "The Study of Temperament through Blood Type" by Tokeji Furukawa.¹ The idea was extended in 1971 after Masahiko Nomi published "Understanding Affinity Through Blood Type."² The term Ketsueki-gata became prominent in Japanese society, the term meaning blood type influences personality.

Blood types are categorized by the ABO Blood Grouping System. This system was discovered by Karl Landsteiner³ in 1901, publishing the A, B, AB and O system. The blood type is determined if an A or B antigen is present on their RBCs. There are 32 recognized blood groups but 4 that are most common as shown in Table 1.^{2,3}

Table 1: This table shows the 4 most common blood types and their antigen/antibody makeup that distinguishes them from other blood types. Differences show the different makeup of each blood type.

Type A: A antigens+ B antibodies
Type B: B antigens +A antibodies
Type AB: AB antigens + no antibodies
Type O: No antigens+ A and B antibodies

According to the American Psychological Association, personality is the different characteristics a person shows in thinking, feeling and behaving.⁴ The most common way to measure personality is "The Big 5" which measures an individual in 5 factors: 1. openness to experience, 2. conscientiousness, 3. extraversion, 4. agreeableness, and 5. neuroticism.⁵

As part of the Minnesota Twin Study, results have shown that personality may have a strong genetic component, as separated twins eventually developed a similar personality to their parents.⁶ Also, empirical studies have consistently shown that

adoptive children show greater resemblance to their biological parents, rather than their adoptive, or environmental parents.⁷ However, research done by Plomin has shown that the predominant influence of personality has been traced back to environment.⁸ Even though twins share genetic material, their surroundings and experiences will shape them into who they are, aside from their DNA shared from their biological parents, if the Minnesota Twin Study was used as an example.

Ever since the publications of the Blood Type + Personality Correlation phenomenon, blood type has become a critical component in the lives of Japanese. Blood type influences job interviews, marriages and the reputation of individuals in society. Businesses in Japan also sell products catered to specific blood types and sports training is developed specifically for each blood type. Discrimination has even occurred in Japan- "bura hara"- based off an individual's blood type and their alleged personality traits. BBC News reports that "In July 2011, Minister for Reconstruction Ryu Matsumoto resigned after being criticized for making insensitive remarks. He blamed his blood type".⁹ Blood type is clearly a prime component in the lives of Japanese people, but does this belief stand scientifically?

Tokeji Furukawa has made his correlation between blood type and personality apparent through highlighting the purported distinct traits that an individual has due to their blood type. Walden University has written about Furukawa's theory as shown in Table 2.¹⁰

Research to test these variables has been published by varying sources including Nawata (2004)¹¹ Wu, Lindsted, Lee (2005)¹² and Tscuchimine, Saruwatari, Kaneda, Yasui-Furukori (2015).¹³ These studies differ from our research as the latest paper has been published more than 5 years ago. In addition,

some results received by the published journals were of mixed consensus. The research was also targeted among Japanese and Taiwanese individuals; cultures where the Blood-Type correlation ideal persists to this day. The present research in this paper includes any nationality, predominantly living in America, which limits the bias that may arise when the participants are surveyed. This signifies they are not mentally predisposed to the notion that blood type and personality are affiliated, which may impact them to answer the survey with the ideal, a belief that the Asian participants may have.

Table 2: This table describes the correlation that Ketsueki-Gata describes, categorizing blood types with different personality traits. The table shows that each type is thought to have different dispositions, which is how individuals are viewed in society.

Type A:	Type B:	Type AB:	Type O:
Earnest, creative, sensible, stubborn, tense	Passionate, active, selfish, irresponsible	cool, controlled, rational, critical, indecisive	confident, strong, cold, unpredictable

■ Methods

Human Participant Research:

As this is a human participant study, there will always be risks even if minimal. For this study, psychological risks were considered as a potential issue. Participants were made aware that this was solely a research study and did not formally indicate any correlation or lack thereof. Risks were minimized by informing participants that they had the right to stop taking the survey at any time; they were also notified that any individual who felt uncomfortable taking a personality assessment or providing their blood type should not participate in this study as they are part of the vulnerable population.

Participants were ages 18+ and gender, race and ethnicity were not considered as identity remained anonymous.

Participants were a combination of individuals in communication with the researcher prior to the study, and individuals who were willing to participate in this survey even if the researcher did not have a connection with them, as the survey had been forwarded from family and friends to others living in their area/others willing to participate. This survey was a text message in which a consent message will be sent to them and a link to complete the survey.

Participants were asked to complete a survey in which they provide their blood type and take a personality test from the International Personality Item Pool, which is described further in the procedures section. This survey took around 1-2 minutes to complete. Participants were not informed of the goal of this study prior to their involvement to limit bias.

Benefits to participants included that they were contributing to a research study aiming to prove/disprove the credibility of the belief that personality is impacted by blood type. They also engaged in retrospective activity as they reflected upon their character traits while completing the survey.

The research was approved by the school psychologist: H. O'Brien, MS, Eds, Psy.D. It was reviewed by the school's Institutional Review Board who concluded that the study posed minimal risk, did not require a written minor assent form, a written parental permission form (as no participant was under the age of 18) and did not require a written informed consent

form for participants older than 18 years of age. No identifiable information was collected. Email collection occurred through the Google Forms software was disabled. The only information received was the date and time the survey was submitted and the participants' responses.

The data will be stored in a secured google forms sheet, for a minimum of 5 years after the study has culminated. The researcher will be the only person with access to results before, during, and after experimentation, as it is her personal Google account with a confidential password.

Procedure:

An online survey was created through Google Forms asking participant about their blood type. The survey questions were based off the International Personality Item Pool (based on the Big 5 Personality Traits: Ocean (Openness to Experience, Conscientiousness, Extraversion, Agreeableness, and Neuroticism)) which also provides the scaling chart to convert responses to numerical values.¹⁴ This pool was created by Lewis Goldberg, which measures the Big 5 Personality Traits and has become the best accepted personality model in modern psychology.⁵ The first question of the survey asked if participant is aware of their blood type, and if answer is no, they were taken to the end of the survey. Otherwise, the participant continued with survey and provided their blood type.

Four questions for each of the five personality types were curated based off OCEAN categories from the International Personality Item Pool. The survey was linked to a Google Sheets page to collect data efficiently and then sent to participants through a text message to 126 individuals on December 5th, 2019. After a span of 2 weeks, the survey page was closed, and data collection and mathematical analysis began. Using the data received from the Google Sheets page from the Google Forms survey, the results were scored using the IPIP (International Personality Item Pool) scaling sheet, and total scores were added for each category of OCEAN. The obtained score for each category was then divided by the highest possible score as determined by IPIP. The scores were then multiplied by 100 to find percentile values and averages were found per OCEAN group.

After conducting the survey, the responses received were analyzed. However, some participants did not know their blood type, and some surveys were not completed in full. After removing these from the database, 100 responses remained. Using the IPIP Scale Score chart, the responses were computed and scaled to numerical values. Each answer from the personality test (strongly disagree, disagree, neutral, agree, strongly agree) was presented with a value from 1-5 depending on the "keyed value" (either positive or negative) of the question. Separated per blood type, the numbers were summed per blood type, placed into their respective OCEAN categories and averaged to find a value. This was repeated for every response until averages for each blood type and its respective OCEAN Category were received.

■ Results

The average values per OCEAN category per blood type were graphed on a bar graph, where slight differences were

apparent per blood type. After, the CHI Square statistical analysis was performed using Microsoft Excel software, as seen in the Figure 1. The probability values (p-values) received were 0.96,0.99,0.99,0.98 and 0.97 for OCEAN respectively. P values range from 0-1 and a higher p value leads to a failure to reject the null hypothesis. The high p values attained from the analysis provide evidence that there is no relationship between blood type and personality and the null hypothesis is failed to be rejected.

According to Table 3, the observed and expected values of the IPIP scores were calculated using CHI Square P Value test. The P value received was 0.98, a very high P value that does not signify a statistically significant value. Table 4, 5, 6, and 7, all show similar values as the P values were in the high 90th percentiles, leading to values that are not statistically significant.

The tables do show some variances from the expected values, and these variances can also be seen through the Figures 3-7. However, the P value test confirms that these values are not statistically significant despite the score fluctuations from the expected values.

Excel P Value Formula
=tdist(x,deg_freedom,tails)

Figure 1: Excel P value formula that was used to calculate blood type/questionnaire P value. P value provides evidence of the need to reject/fail to reject the null hypothesis.

Blood Type and Personality Probability Values: Comparing Observed vs Expected for an OCEAN category:

Table 3: This table displays the extraversion questions compared with blood type. P value using observed and expected values resulted to 0.99, a large value leading to the failure of rejecting the null hypothesis. There is no relationship between blood type and extroversion.

Blood Type	Observed	Expected	P Value
A-	2.68	3	0.9883752
A+	4.125	3	
B+	3.5	3	
AB+	3.25	3	
O-	3.3	3	
O+	2.93	3	

Table 4: This table displays the agreeableness questions compared with blood type. P value using observed and expected values resulted to 0.98, a large value leading to the failure of rejecting the null hypothesis. There is no relationship between blood type and agreeableness.

Blood Type	Observed	Expected	P Value
A-	2.68	3	0.9883752
A+	4.125	3	
B+	3.5	3	
AB+	3.25	3	
O-	3.3	3	
O+	2.93	3	

Table 5: This table displays the conscientiousness questions compared with blood type. P value using observed and expected values resulted to 0.99, a large value leading to the failure of rejecting the null hypothesis. There is no relationship between blood type and conscientiousness.

Blood Type	Observed	Expected	P Value
A-	3.5	3	0.9892896
A+	3.97	3	
B+	3.43	3	
AB+	3.25	3	
O-	3.3	3	
O+	3.43	3	

Table 6: The table displays the neuroticism questions compared with blood type. P value using observed and expected values resulted to 0.97, a large value leading to the failure of rejecting the null hypothesis. There is no relationship between blood type and neuroticism.

Blood Type	Observed	Expected	P Value
A-	3.5	3	0.9736089
A+	4.19	3	
B+	2.9	3	
AB+	3.83	3	
O-	3.41	3	
O+	3.15	3	

Table 7: The table displays the openness to experience questions compared with blood type. P value using observed and expected values resulted to 0.96, a large value leading to the failure of rejecting the null hypothesis. There is no relationship between blood type and openness to experience.

Blood Type	Observed	Expected	P Value
A-	3.75	3	0.9613414
A+	4.13	3	
B+	3.7	3	
AB+	3.5	3	
O-	3.67	3	
O+	3.13	3	

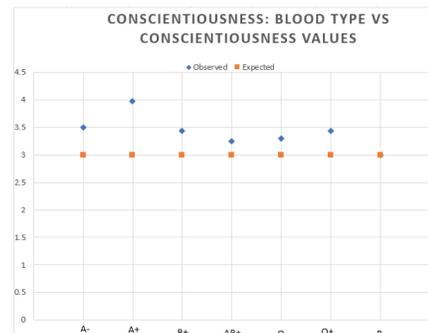


Figure 2: This graph illustrates the Conscientiousness IPIP Scores for each blood type. This graph shows deviations from the expected values, with scores being higher for all blood types in regard to conscientiousness.

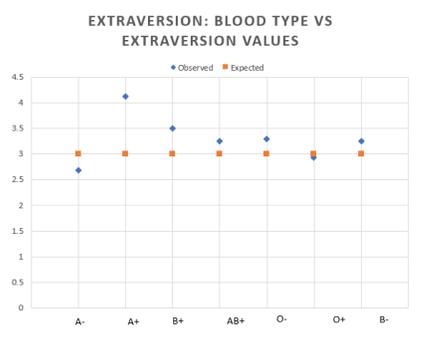


Figure 3: This graph illustrates the Extraversion IPIP Scores for each blood type. This graph shows deviations from the expected values, with scores being higher for all blood types except A- and O+ in regard to extraversion.

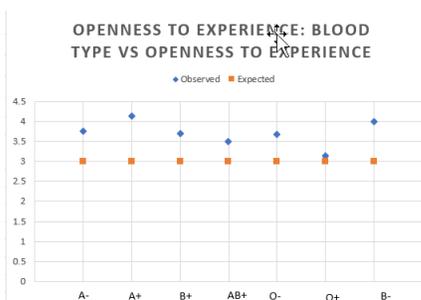


Figure 4: This graph illustrates the Openness to Experience IPIP Scores for each blood type. This graph shows deviations from the expected values, with scores being higher for all blood types in regard to openness to experience.

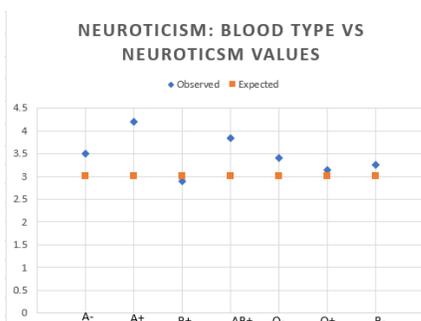


Figure 5: The graph identifies the neuroticism IPIP Scores for each blood type. This graph shows deviations from the expected values, with scores being higher for all blood types except B+ in regard to neuroticism.

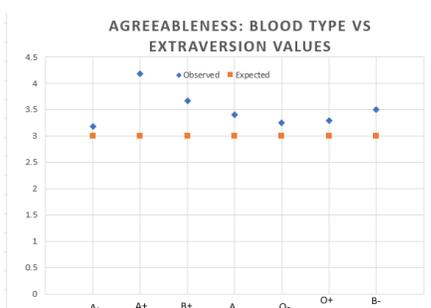


Figure 6: This graph illustrates the Agreeableness IPIP Scores for each blood type. This graph shows deviations from the expected values, with scores being higher for all blood types in regard to agreeableness.

Conclusion

Through the statistical analysis of 100 adults, using their blood types and personality questionnaire responses, a correlation was not found between blood type and personality. The P values received from the Excel Software CHISQUARE test were in the high 90th percentiles, and a result under 5% would be considered statistically significant, whereas this result is not significant. Although the scatterplot shows deviations from expected values for all blood type-personality characteristic combinations, the CHISQUARE test does not support any correlation. The high P value failed to reject the null hypothesis "The blood type of an individual has no impact of their personality traits as represented by openness, conscientiousness, extraversion, agreeableness, and neuroticism".

The overall hypothesis has been supported by the results. Personality may have a genetic component, as seen in the Minnesota Twin Study, but is not dependent on an individual's blood type. It may, however, be influenced by external forces, such as environment.¹⁵ Personality tends to be molded by experiences and events that a person has been through, rather than a strict biological component. Standards, such as profiling humans according to blood type, as seen in Japan, set individuals into a mold they cannot escape, which is unreasonable as shown by studies done in Japan and once again through this study, which focuses on all participants regardless of their background.¹¹ Blood type should not denote the "expected" characteristics of an individual. Although genetic components may have an impact, personality is not dependent on blood type as can be seen from this study and the results received.

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Student Perspectives on the Challenges Faced During the Transition to High School

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ABSTRACT: The transition from middle school to high school is a pivotal time in the life of a student and has the potential to be quite stressful. The goal of this study was to evaluate the concerns of ninth-grade students when entering high school. An anonymous web-based survey regarding their experiences with the transition from middle school to high school was distributed to twenty-one ninth-grade students from a high school in Boston, MA in April 2019. A total of 21 of 24 surveys were completed (87.5% response rate). The most significant student concern related to their high school transition was the ability to manage their time, followed by increased academic expectations, greater amount of homework, adjusting to new classmates, making new friends, and adjusting to new teachers. For improving transition to high school, students proposed more personalized support in the early phase of the transition to high school and better communication between parents and school counselors in order to identify and address student concerns in a timely fashion. Therefore, teachers, counselors, and parents should work together in identifying strategies to help students entering high school.

KEYWORDS: Behavioral and Social Sciences; Clinical and Developmental Psychology; High School Transition; High School Counselors; High School Teachers.

Introduction

The transition from middle school to high school has the potential to be stressful. High school is a time when many ninth graders find themselves lost in an unfamiliar, less personal environment, struggling to keep up with the demanding coursework that was not as present in middle school.¹ Research has shown that students who experience a positive transition and successful freshman year, are more likely to have a successful overall high school experience.² On the other hand, students that do not have a successful transition into high school are more likely to become discouraged and eventually drop out of school.³ Indeed, studies suggest that approximately one-third of the recent high school dropouts never were promoted beyond ninth grade.⁴ The goal of this study was to evaluate the concerns of ninth-grade classmates during their transition from middle school to high school.

Methods

An anonymous web-based survey regarding high school freshmen's experience with the transition from middle school to high school (Table 1) was distributed to twenty-four ninth-grade students from a high school in Boston, MA in April 2019. Students were in a different classroom than that of the author and the survey was approved by the dean of the freshmen class. The survey was completed, and the information was analyzed to determine the concerns raised by the students entering high school.

Results and Discussion

A total of 21 of 24 surveys were completed (87.5% response rate) by both female [42.9% (9/21)] and male [57.1% (12/21)] participants.

Table 3: Survey questions.

1. What is your gender?
 - a. Female
 - b. Male
 - c. Other:
 - d. Prefer not to say
2. What middle school did you graduate from?
3. What are the most important challenges that you faced during the transition to high school?
 - a. Adjusting to new classmates
 - b. Adjusting to new teachers
 - c. Making new friends
 - d. Academic expectations
 - e. Amount of homework
 - f. Ability to manage my time
 - g. Other:
4. What suggestions (if any) do you have for improving the transition to high school?

The most significant student concern related to their high school transition was the ability to manage their time (31%), followed by increased academic expectations (24%), greater amount of homework (17%), adjusting to new classmates (10%), making new friends (10%), and adjusting to new teachers (7%).

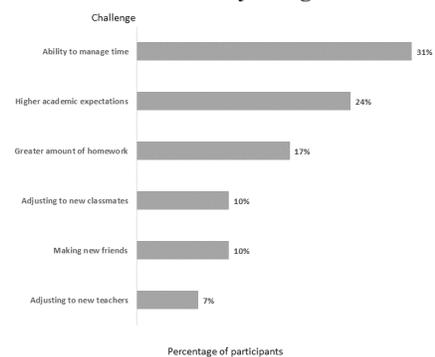


Figure 1: Challenge faced by students during their transition to high school.

When asked about suggestions for improving the transition to high school, students proposed more personalized support in the early phase of the transition to high school and better communication between parents and school counselors in order to identify and address student concerns in a timely fashion. The responses were comparable when stratified by gender and the name of middle school.

The results of the study show that the most significant challenge experienced by students during their transition to high school is managing their time while trying to adapt to increasing academic expectations and workload. This is in line with studies showing that academic concerns are the most prominent during the transition to high school,⁵ followed, similar to our study, by social concerns (making new friends and fitting in),⁶ and procedural concerns (becoming familiar with the new teachers, new school infrastructure, and the new schedule).^{7,8}

The ninth-grade students participating in the survey suggested more personalized support during the onboarding to high school and improved communication between parents and school counselors in order to identify and address student concerns in a timely fashion.

The crucial role of school counselors in supporting students during their transition to high school is well recognized.⁹ The American School Counselor Association (ASCA) has developed best practices guidelines that school counselors follow to help students overcome the challenges of high school transition.¹⁰ School counselors could further improve the middle-to-high school transition experience of their students by performing a more in-depth intake assessment of their students in order to identify students that would benefit from an earlier support.⁹ Furthermore, in order to provide a more comprehensive understanding of the issues that students face when entering high school, close communication between counselors, teachers, and parents is very important.

Parent involvement is essential for a successful transition to high school. Indeed, studies show that when parents work closely with teachers and remain involved in the education of their high-school children, ninth graders have a more successful experience.⁶ Importantly, the students participating in this study recognize the importance of parent involvement for their transition to high school. They would welcome better communication between their parents and counselors, both early, during the onboarding process, and throughout the ninth grade.

The present study has some limitations. First, the small sample size could limit the ability to generalize the findings. Surveying a larger group of high school ninth grade students from multiple institutions would be an important next step in generalizing and validating the findings of the study. Second, although the response rate was very high (87.5 %), meaningful differences between students who answered the questionnaire, and the non-respondents cannot be excluded. Third, a ranking scale rather than the “best answer” question format in would have better assessed the perspectives of the students on challenges faced during their transition to high school. Fourth, a pre- and post- transition survey would have better assessed

transition challenges and provided insights on the transition expectations of middle school students.

Even with these limitations, the findings provide meaningful insights into the challenges faced by students during their transition to high school.

■ Conclusion

The transition to high school is a critical period for students. The results of the survey show that improved transition support is needed for the incoming high school freshmen. Teachers, counselors, and parents should work together in identifying strategies to help students entering high school.

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Characterization, Treatment, and Recovery of Chemical Species from the Bayer Process' Red Mud Waste

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ABSTRACT: Aluminum is an indispensable metal nowadays. It can be used as a raw material for several industrial products. In order to obtain aluminum, it is necessary industrial processing that involves the extraction of bauxite ore, its refining, and the consequent obtaining of alumina, that is later converted into metallic aluminum. In addition to producing alumina, the process generates a significant amount of a waste called "red mud", which represents a challenge for the industries that need to dispose it without harming the environment. Therefore, this study's main objective was to characterize a sample of red mud from Companhia Brasileira do Alumínio (CBA). The residue was characterized in terms of chemical composition, mineralogical composition, as well as leaching and solubilization abilities. The waste showed a predominance of the following compounds: Fe_2O_3 , Al_2O_3 and SiO_2 . In the solubilization test, the sample presented a concentration of some elements above the limit allowed by environmental standards. In the leaching test, all parameters were below the standard's limits. In the red mud treatment stage, the alkalinity was successfully removed after three washes with distilled water. The subsequent steps of iron and aluminum recovery, and separation of the rare earth elements will be carried out.

KEYWORDS: Chemistry; Environmental Chemistry; Red Mud; Materials Characterization; Recovery and Recycling.

■ Introduction

Aluminum is the third most abundant chemical element on planet Earth.¹ It is a bright, malleable, resistant metal with multiple uses. The production of this material is indispensable in today's age as it can be used as raw material for several industrial products, including food and beverage packaging, car wheels, building materials, aircraft and cell phones parts, pans, wheelchairs, benches, stairs, chairs, etc. As it has several applications, aluminum production is gradually increasing worldwide. However, to obtain aluminum as a final product, solid waste is generated, which, if not treated or disposed of properly, can lead to severe environmental problems.

Industrial processing is necessary to obtain aluminum. It involves the extraction of bauxite ore, its refining through the Bayer Process, and the consequent obtaining of alumina (Al_2O_3). Later on, the alumina is converted into metallic aluminum. However, the Bayer Process, in addition to producing alumina, also generates a significant amount of a waste called red mud (RM). RM represents a considerable problem for the refining industries, because of the harm that its inappropriate disposal can cause to the environment. Usually, the red mud is placed in dams (Figure 1). However, this type of structure represents a risk due to the possibility of rupture. This kind of accident has already happened in Brazil in Mariana city in 2015 and Brumadinho city in 2019.

Brazil has a series of laws, which contains rules to prevent and reduce the generation of waste in the country. It also encourages the reuse of solid waste that has economic value and can be recycled. Also, this measure establishes the appropriate environmental disposal of waste that cannot be recycled or reused.²



Figure 1: Site where the red mud is placed after the Bayer Process.

The red mud generated in the Bayer Process is an insoluble residue composed of aluminum hydroxides, iron oxides and hydroxides, kaolinite or halloysite, titanium oxide, and other elements.³ This waste is classified as highly corrosive by the Associação Brasileira de Normas Técnicas (ABNT).

Some researchers have studied applications for red mud, such as adding it to the composition of ceramic materials, the manufacture of cement, bricks, and using it for alkalizing acid soils, etc. However, these studies do not have large-scale applications, and therefore, the waste generated is not fully used. Besides, these uses do not lead to enough profit to attract the attention of pertinent companies.⁴

For a long time, red mud was considered an unusable waste for the aluminum industry. However, economic expenditures and environmental threats related to the disposal of this waste have encouraged companies and scientists to search for more environmentally safer options, which provide a reduction in the amount of RM destined for dams.

Research aimed at recovering iron, titanium, and other metals have been developed. Generally, the studies presented in the literature are not financially viable.⁴ However, if the recovery of

more valuable elements, such as lanthanum and cerium, which are rare earth elements, become possible, the commercialization of these products can most likely cover the costs for the treatment of the mud, making the process viable from an economic point of view.

The rare earth elements are essential chemical elements for the production of catalysts, magnets, luminescent materials, among other products. Annually, their consumption around the world reaches approximately 150 thousand tons.⁵

Currently, China has a global monopoly in the production and sale of rare Earth elements, earning about 5.7 billion dollars a year and administrating 95 % of the worldwide reserves. Many companies fear an increase in the prices of rare earth elements, as it had already occurred in 2010, when these compounds reached the highest level so far, and there are no other alternative resources to replace their functions by industry.⁶

Although these components are in abundance on earth's crust, they are complicated to extract, leading their prices to be increased on the world market.⁶ Therefore, developing a process in stages to recover valuable resources from the RM can be an exciting alternative for companies that produce aluminum to generate profit and make the disposal of this residue less hazardous for the environment.

The main objective of this research is to analyze red mud and develop a method to recover a considerable number of compounds and chemical elements, including producing an alkaline solution of NaOH, Fe, Al, La, and Ce, from it. Hence, the companies that generate this residue can reduce its quantity and furthermore grow economically.

■ Methods

Sample collection:

A sample of the red mud was collected in February 2018 at Companhia Brasileira de Alumínio, which uses the Bayer Process to obtain alumina. CBA is located in the city of Alumínio, close to São Paulo city, in Brazil.

The red sludge is generated in a step called "Sludge Filtration." In this stage, the waste is separated from the process with a filter.

Chemical, morphological and environmental characterization of the waste:

The collected red mud was characterized in terms of chemical composition by X-ray fluorescence analysis (Table 1). The equipment used was the PANalytical X-ray fluorescence spectrometer, model Zetium. The Loss on Ignition (LOI) was carried out at 1,020 °C for two hours.

The morphology of the RM residue was verified in a Scanning Electron Microscope model Philips XL-30, using increments of 250 and 1500 x at 15 kV (Figure 2). The samples were placed on a support and covered with a thin layer of gold to make them conductive before being subjected to the microscope. Leaching and solubilization tests were performed according to the Brazilian law standards, called "Associação Brasileira de Normas Técnicas" - ABNT (Tables 2 and 3).

Alkalinity Removal:

The experimental part of this stage was performed at the chemistry laboratory of the Dante Alighieri high school. First, the RM was dried at 1050 °C in an oven at night (Figure 3).

the pH of the mixture was retested. Then, again, the residue was stirred for 30 minutes followed by rest intervals of 15 minutes. The pH measurements were performed two more times so that the pH variability could be verified.

Table 1: Chemical analysis of the red mud.

Component	Content %
Fe ₂ O ₃	30.3
Al ₂ O ₃	22.0
SiO ₂	15.4
Na ₂ O	8.57
CaO	4.96
TiO ₂	3.03
SO ₃	0.90
P ₂ O ₅	0.62
PbO	0.17
K ₂ O	0.16
Cl	0.15
ZrO ₂	0.15
ZnO	0.13
MgO	0.11
MnO	0.09
Cr ₂ O ₃	0.05
CeO ₂	0.04
CuO	0.03
SrO	0.03
BaO	0.03
La ₂ O ₃	0.02
Nb ₂ O ₅	<0.01
LOI	13.0

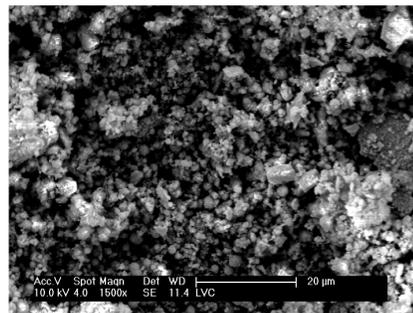


Figure 2: The morphology of the RM residue verified in a Scanning Electron Microscope, using increments of 1500X.

Table 2: Leached extract of RM.

Parameter (mg/L)	Limits for Quantification (LQ)	Results	Brazilian law standards
Total of Arsenic	0.010	<LQ	1.000
Total of Barium	0.010	<LQ	70.000
Total of Cadmium	0.005	<LQ	0.500
Total of Lead	0.01	<LQ	1.00
Chromium	0.010	0.154	5.000
Fluorine	0.10	<LQ	150.00
Total of Silver	0.010	<LQ	5.000
Total of Selenium	0.010	<LQ	1.000
Total of Mercury	0.00100	<LQ	0.10000

Table 3: Solubilized extract of RM.

Parameter (mg/L)	Limits for Quantification (LQ)	Results	Brazilian law standards
Total of Aluminium	0.010	287.600	0.200
Total of Arsenic	0.010	<LQ	0.010
Total of Barium	0.010	<LQ	0.700
Total of Cadmium	0.005	<LQ	0.005
Total of Lead	0.010	<LQ	0.010
Total of Cyanide	0.005	0.009	0.070
Chloride	0.50	23.17	250.00
Total of Copper	0.010	<LQ	2.000
Total of Chromium	0.010	0.758	0.050
Total of Iron	0.010	<LQ	0.300
Fluoride	0.10	<LQ	0.80
Phenol Index	0.03	0.59	0.01
Total of Manganese	0.010	<LQ	0.100
Nitrate	0.11	<LQ	10.00
Total of Silver	0.010	<LQ	0.050
Total of Selenium	0.010	<LQ	0.010
Total of Sodium	1.00	1470.00	200.00
Sulfate	0.50	36.95	250.00
Surfactants	0.10	1.06	0.50
Total of Zinc	0.010	<LQ	5.000
Total of Mercury	0.00100	<LQ	0.00100

**Figure 3:** Sample being subjected to a muffle.**Figure 4:** PH universal indicator.**Figure 5:** Samples being agitating.

After these steps, the residue was filtered through a beaker with filter paper and funnel (Figure 6). The sludge was subjected to a drying process at 105 °C, and its mass was determined on an analytical balance. After weighing, the sample was heated in a furnace for one hour at 600 °C.

**Figure 6:** Residue being filtered through a beaker with filter paper and funnel.

Iron removal:

The methodology for this step was carried out at the Dante Alighieri high school laboratory, and the parameters used were adapted from the literature according to Aguirre and Diaz.⁷ First, calculations to define the molar concentration of sulfuric acid were done. Thus, approximately 20 mL of diluted sulfuric acid (already prepared), was added to a 100 mL beaker containing 4 g of heavy calcined red mud powder on a precision scale.

The mixture was subjected to a magnetic stirrer previously heated to 80 °C for 1 hour. During that process, it was noted the release of gas and the clarification of the mixture (Figure 7). After stirring, the suspension was filtered (Figure 8). The methodology flowchart is summarized in Figure 9.

**Figure 7:** Red mud in a magnetic stirrer being heated.



Figure 8: Filtration system.



Figure 9: Photographs taken at the Laboratório de Reciclagem, Tratamento de Resíduos e Extração (LAREX).

Alkalinity removal:

The initial pH of the laboratory distilled water was 6. The first step was adding 100 mL of water to the alkaline RM, then the suspension went to the magnetic stirrer. Later on, the mixture was left to rest. The pH of the water measured was 10. In the subsequent stirring, there was no change in pH, which remained at 10.

The sample mass, after the wash, was 9.4 g, and the volume of the filtered solution, 80 mL. The pH of the duplicate wash water was 10. The mass of RM after the removal of alkalinity was 9.35 g, and the volume of the filtered solution, 75 mL. Therefore, the alkalinity removal step was valid since the red mud washing water reached pH 10.

■ **Results and Discussion**

Alkalinity removal:

As shown in Table 1, the red mud has a predominance of the following elements: Fe_2O_3 , Al_2O_3 , SiO_2 , Na_2O , CaO , and TiO_2 . This result is similar other authors characterization.^{4,8,10}

Figure 2 shows the residue morphology by scanning electron microscopy, with a 1500X magnification. The compounds in red mud have different sizes and shapes.

The results of the leaching and solubilization tests are shown in Tables 2 and 3, respectively. The ABNT standard defines leaching as the process for determining the transfer capacity of organic and inorganic substances present in solid waste (ABNT, 2004). The waste is classified according to its behavior in contact with a solvent. It can be classified as hazardous or non-hazardous waste. In the leaching test (Table 2), all parameters were below the limits defined by the law

(ABNT NBR 10005: 2004 standard), therefore the waste is classified as non-hazardous.

From the analysis of the solubilization test (Table 3), it can be concluded that some of the sample elements show a concentration above the required limit: total aluminum, total chromium, phenol, and total surfactants. Therefore, the waste is classified as non-inert.

According to the ABNT Standard, hazardous wastes are classified into class I, while non-hazardous wastes are in class II. Non-hazardous waste can also be classified into two subtypes: class II A (non-inert) or class II B (inert). Therefore, the red mud is classified as class IIA.

Red Mud Treatment:

At this stage of the project, we explored a proposal for the treatment of red mud that aimed to recover compounds present in it. According to several studies, rare earth metals are usually extracted from another compound using acidic solutions, such as sulfuric and hydrochloric acids.^{4,7} The red mud is alkaline and, therefore, this alkalinity could hinder the extraction of rare earth metals. Consequently, the alkalinity of the residue should be the first to be removed. The alkaline solution could return to the Bayer Process, as it is one of the raw materials used in this process.

Because the red mud has a high content of the elements iron and aluminum oxides (as shown in Table 1, with percentages of 30.3 % and 22.0 %, respectively), the next steps should be the extraction of these two elements. The recovered iron can be sold, and the aluminum oxide returned to the Bayer process. After these initial stages, the final step is the extraction of rare earth metals, which are rated with a high commercial value.

■ **Conclusion**

The red mud remaining from the Bayer process presented Fe_2O_3 , Al_2O_3 , SiO_2 , Na_2O , CaO , and TiO_2 as main components. In the solubilization test, the sample showed a concentration of aluminum, chromium, phenol, sodium, and surfactants above the standard. In the leaching test, all parameters were below the limits defined by the Brazilian standard for residues. Therefore, the red mud was classified as class IIA waste.

The alkalinity removal of the red mud was successfully performed after three washes with distilled water. However, the subsequent steps of iron recovery, extraction of aluminum, and separation of rare earths present in the waste will still be carried out. This part of the methodology was not executed in 2020 due to restricted access in the laboratories of Dante Alighieri High School.

The next steps of this study will be to carry out the experimental part pending in the partner laboratory of the project (LAREX - USP) and to present this proposal for the recovery of resources for the company that generates red mud.

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The Effect of Global Warming and Ocean Acidification on *Halichondria panicea* Bacteria

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ABSTRACT: Marine Sponges are becoming an increasing source of novel biomedical and antibacterial compounds. Many of these compounds are synthesized as secondary metabolites from symbiotic bacteria and have immense potential in the pharmaceutical industry. However, climate change may pose a threat to the viability of marine sponges and result in the loss of future medical discoveries. Therefore, this paper looks at the effect climate change may have on marine sponges by subjecting fragments of the marine sponge, *Halichondria panicea*, into aquaria representing different climate change scenarios to study the effect that global warming and ocean acidification may have on its symbiotic bacteria. To model climate change towards the end of the 21st century, conditions from the IPCC's 2014 climate change report were simulated to determine specific growth conditions. The fragments were placed in the different RCP growth conditions for two weeks, then dissociated, filtered, and the extracts incubated on Hektoen enteric agar for 48 hours. The results showed that climate change has adverse effects on the marine sponge, *Halichondria panicea*, by decreasing their symbiotic bacterial population by around 18 %.

KEYWORDS: Earth and Environmental Sciences; Environmental Effects on Ecosystems; Marine Biology; Climate Change; Marine Sponges.

Introduction

Some forms of climate change have been modeled and discussed by the IPCC (Intergovernmental Panel on Climate Change). In doing so, they have created future scenarios (RCPs) detailing the rate of global warming and ocean acidification. These scenarios are based on the possible actions of humanity and how their intervention can change the course of climate change.¹ An RCP number of 2.6 (RCP2.6) means increased human intervention and lowered ocean temperatures and stabilized pH levels. As the number increases (6.0, 8.5), so does ocean acidification and global warming, meaning that humans are taking little part in efforts to reduce carbon emissions and ocean acidification. These models show that the greater the projected changes are, the more stable and hospitable the environment will become.² These RCP scenarios and their modeled effects are shown in Figure 1.

In a single liter of ocean water, there are over 1 billion individual bacterial cells and 10 billion viruses. Sponges, often referred to as the filters of the ocean, process over 50,000 times their volume of this pathogenic seawater in a day.³ As they are in continual contact with this endless stream of unique microorganisms, sponges begin to develop complex antibacterial compounds. Sponges attain these medical compounds through their symbiotic relationship with certain bacteria. Many times, when these bacteria are stressed, they produce a substance often referred to as a secondary metabolite. The molecules are highly specialized and are created to target specific problems.⁴ In the case of the marine sponge, *Halichondria panicea*, current research has shown that there are a variety of symbiotic bacteria living within the organism. However, the sponge's main symbiotic bacteria belong to the genus *Rhodobacter* (Proteobacteria, subdivision α).⁵ These bacteria are gram-negative and possess

immense potentials in the pharmaceutical industry as they are able to synthesize highly complex antibiotics and other drugs.⁶ Since these bacteria are relatively small, 0.6–0.8 microns long, a 1-micron syringe filters were used to purify sponge extracts.⁷

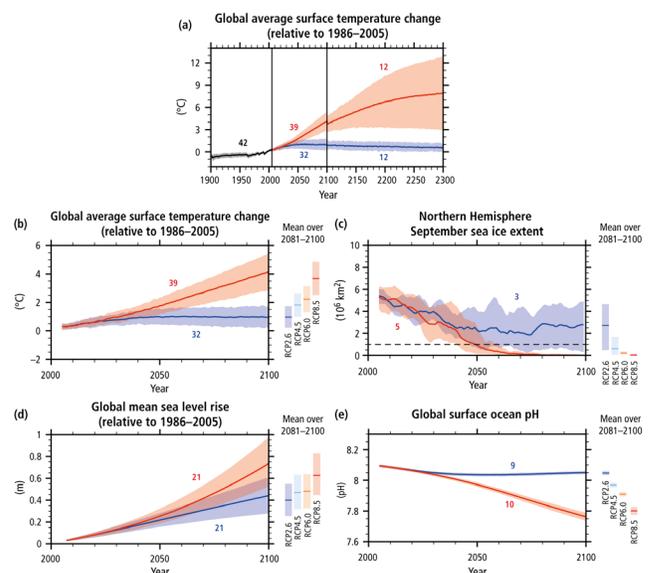


Figure 1: Projected effects of increases in atmospheric CO₂ concentration by RCP scenarios 2.6, 4.5, 6.0, and 8.5 plotted as: a) surface temperature change, b) glacier melting, c) sea level rise, and d) ocean pH decline. Each RCP scenario, represented by the different colored projections, will have varying effects on the Earth's environment, however the most severe of which will increase global temperatures by about 4°C and decrease ocean pH levels by about 0.3 units by 2100.²

Although some researchers have shown that sponges may transcend climate change, little is known about its bacteria, leaving much to be imagined for a sponge's medical potential

in future scenarios.⁸ Therefore, the understanding of climate change as it relates to bacteria, is the first step towards understanding what possible challenges may lie in the future as it relates to the viability of marine sponges and the pharmaceutical industry.⁹

■ Methods

The *Halichondria panicea* sponges were raised in 2-gallon aquaria for the duration of two weeks and then subjected to different temperature and pH levels representing present and possible future conditions. After two weeks, the bacteria living in the sponges were sampled using standard techniques and were tested for viability. About 20 grams (wet weight) of *Halichondria panicea* sponge was collected from boat docks near the Cabrillo Marine Aquarium. They were then cut into 16 pieces, each measuring about one cubic centimeter, and placed into 16 different tanks (batch system). The tanks were separated into groups of 4 labeled trial 1, trial 2, trial 3, and trial 4. Each tank within each trial was then labeled control, RCP 2.6, RCP 6.0, and RCP 8.5 and subjected to different water temperatures and pH levels based on their specific conditions shown in Table 1. The temperature in each of the test tanks were increased utilizing an aquarium heater and the pH decreased utilizing a soda streamer. To reduce the pH levels, CO₂ was bubbled into artificial seawater and then the pH levels were manually adjusted by changing the proportion of unaltered seawater to CO₂ streamed seawater. After 2 weeks, the sponges were washed with sterile artificial seawater, and the tissue was homogenized using a pestle and mortar. The extracts were then filtered through a 1micron syringe filter, diluted to 10:2 concentration with artificial sea water and inoculated onto Hektoen enteric agar plates for 48 hours with a 100 µL micropipette. After the incubation, the number of colonies on each plate were counted three times to derive a mean (to ensure consistency). Each tank had LED lighting strung above, cycling at 12-hour intervals. Each tank also had a heater used to alter temperatures, a Fluval AquaClear Power Filter with activated charcoal and sponge filtration media, and a five-watt Hydor Koralia Circulation pump. The sponges were spot-fed once a day with 5 mL of Brightwell Aquatic's PhytoGreen-M phytoplankton suspension. A 10 % water change was also conducted on the tanks every three days for the duration of the two-week testing period.

Table 1: Treatment group for each RCP scenario depicts temperature increase and pH decrease per RCP treatment group.

Treatment Groups Water Conditions				
	Control	2.6	6.0	8.5
Temperature Increase (C):	0	1	2.5	4
pH Decrease:	0	0.07	0.2	0.3

■ Results and Discussion

The number of viable bacterial colonies from each plate are given in Table 2. A One-way ANOVA test was performed on the raw data utilizing Excel Spreadsheets and yielded a P-value of 0.0223 (depicted in Figure 2). After the initial significance

value was determined, a Tukey Post-hoc test was performed to determine which of the treatments were significantly different. It showed that there was statistical significance between the control and RCP 8.5 and RCP 6.0 treatments (depicted in Figure 3).

Table 1: Results showing the number of viable bacteria colonies from each treatment group.

Number of Viable Bacteria Colonies Per Trial				
	Control	2.6	6.0	8.5
Trial 1	77	77	60	55
Trial 2	92	79	68	70
Trial 3	83	70	67	63
Trial 4	74	69	72	74

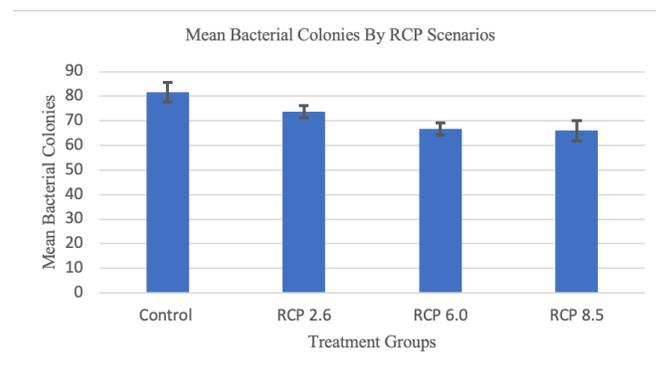


Figure 2: Graphed results from the one-way ANOVA statistical test shows the average number of viable bacteria colonies found on agar dishes after each treatment. There is a general decreasing trend in the number of viable colonies as RCP scenarios worsen; the treatment groups are also proven to be statistically different by the resulted P-value of 0.0223.

treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	2.3095	0.3989372	insignificant
A vs C	4.3956	0.0392897	* p<0.05
A vs D	4.6191	0.0298164	* p<0.05
B vs C	2.0860	0.4814655	insignificant
B vs D	2.3095	0.3989372	insignificant
C vs D	0.2235	0.8999947	insignificant

Figure 3: Results from the Turkey post-hoc test: A represents control treatment, B represents RCP scenario 2.6 treatment, C represents RCP scenario 6.0 treatment, and D represents RCP scenario 8.5 treatment.

From this data analysis, it was found that there was a modest decrease (from 10 to 20 percent) in bacterial colony numbers relative to the control for all treatments, however, the decrease was statistically significant only for the conditions representing scenarios 6.0 and 8.5. The mean number of viable bacteria colonies from the control treatment was about 82 colonies; the mean number of viable bacteria colonies from the RCP 2.6 treatment was about 74 colonies; the mean number of viable

bacteria colonies from the RCP 6.0 treatment was about 67 colonies; the mean number of viable bacteria colonies from the RCP 8.5 treatment was about 66 colonies.

There is considerable variation among the replicates. The reason for this may be a result of human error, however, the specifics are largely unknown. One possible reason for variation is the difference in the population density of microbes within the sponge. Since each individual sponge fragment was only measured and not weighed, this could have resulted in some fragments having a larger population of microbes at the beginning of the experiment, affecting the results.

Although there was a larger proportion of viable bacteria from the control treatment when compared to the RCP treatments, this does not indicate causation. The one-way ANOVA test revealed that there were significant mathematical variations among the treatments. The specific treatments which were different were revealed by the Turkey Post-hoc test to be RCP treatments 6.0 and 8.5 when compared to the control treatment.

Although bacteria from the marine sponge, *Halichondria panicea*, were inoculated onto agar plates and counted for colonies, it cannot be certain that the bacteria were from the genus *Rhodobacter* (Proteobacteria, subdivision α). Due to limitations in equipment, the bacteria's DNA sequence was not identified and compared to pre-existing records; however, steps were taken to increase the chances of viable colonies from *Rhodobacter* (Proteobacteria, subdivision α). These steps include inoculation onto a selective gram-negative plate and filtration with 1-micron syringe filters.

■ Conclusion

RCP scenarios 6.0 and 8.5 are statistically different from the control treatment according to a Tukey post-hoc test conducted on the raw data. The results suggest that if climate change continues along a 6.0 or 8.5 pathway, bacteria within marine sponges may be significantly affected. Recent research has shown that this may already be happening to certain symbiotic bacteria within corals. As oceans are heating up, symbiotic microbes are developing temperature-induced necrosis, which threatens their ecosystems' stability.¹⁰

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George Hasnah is a dedicated and passionate high school researcher. He hopes to peruse a career in microbiology and is a devoted activist in the fight against climate change. Through his passions, he has started several environmental clubs at his school and a non-profit organization, helping students in STEM.

Implementation and Governance Issues in the Water-Energy-Food Nexus

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ABSTRACT: With the increasing demand for water, food, and energy in the next few decades, the water-energy-food (WEF) nexus was created to examine the synergies between these key sectors and how they will be implemented in different communities. This paper reviews the current literature on nexus governance and local implementation. This review suggests that a strong understanding of the interconnectedness between the water, food, and energy sectors among decision makers, consideration of values and interests of local stakeholders, and availability of political support are essential for successful implementation of the WEF nexus model at the local level. Decentralized projects also contribute positively to households and local livelihoods. Through several global examples, this paper lists literature-based examples and case studies of how sustainable, rural livelihoods can be promoted with the nexus framework.

KEYWORDS: Water-Energy-Food Nexus; Implementation; Decentralized; Livelihoods; Governance.

■ Introduction

Adequate food supply, modern energy services, and access to safe drinking water are essential for reducing poverty and moving toward sustainable development. The demand for these basic needs is expected to increase due to rapid population growth, economic development, and urbanization.¹ Many countries, including South Africa, Ethiopia, Mexico, Brazil, India, and Nepal, have been exploring solutions to water scarcity and overconsumption.¹ Jay Famiglietti, senior water scientist at the National Aeronautics and Space Administration (NASA), has cautioned that “the water table is dropping all over the world [and] there’s not an infinite supply of water.”² The Economist reported that water disputes lead to increased international tension.³ Moreover, in its 2011 publication entitled *Water Security: The Water-Food-Energy-Climate Nexus, the World Economic Forum* emphasized that water has been continuously underpriced, groundwater has been exhausted, and that, unlike energy, water has no alternatives or replacements.⁴ Sustaining economic growth in the face of water scarcity and increasing demand for food, water, and energy is one of this century’s greatest challenges.

Both water and energy are needed to produce food; energy is required in all phases of water consumption; water is used to make energy. The intricate connections between these three resources were used to create the water-energy-food (WEF) nexus. The WEF nexus was introduced by the 2011 World Economic Forum to “help decision makers better understand risks associated with managing resources ahead of time and respond proactively in times of crisis.”⁴ The Food and Agriculture Organization states that “the [WEF] Nexus has emerged as a useful concept to describe and address the complex and interrelated nature of our global resource systems, on which we depend to achieve different social, economic and environmental goals.”⁴ The word nexus means “to connect” and is used to

describe the interdependencies and trade-offs between water, energy, and food resources and how they are managed. The WEF nexus’s goal is to understand its three different sectors under one complex system. There have been several concerns about the WEF nexus’s precise meaning. Some authors claim that the term is seen as a buzzword, or a word that gains prominence due to “a combination of ambiguous meaning and strong normative resonance.”⁵ Others report that many countries are not aware that the WEF nexus exists, suggesting that the nexus must be adapted beyond the academic domain.⁵

A conceptual framework of the novel WEF nexus debuted on the international stage at the 2008 World Economic Forum. Its introduction encouraged pivotal research on linkages between water and energy to sustainable development and policy integration.¹ Specifically, WEF emerged as a security concept in light of resource scarcity after the energy and food crisis in 2007 and 2008.¹ The WEF nexus has since been used to analyze specific issues like ethanol production, bioenergy, and irrigation. South Korea and Indonesia use the WEF nexus to simulate nationwide resources, plan urban water systems, and develop agricultural plans. Many African countries are using the nexus to advance renewable energy transition plans.⁶

Even so, the WEF nexus is far from perfect and critics worry about its ability to adjust to different contexts. An article published by the *Food and Agriculture Organization* at the United Nations High-Level Political Forum 2018 states that “limited information undermines evidence-based decision-making. This is due to the frequent lack of reliable and up-to-date data on the status of the WEF sectors at local level but also often at national level, in particular in developing countries,” and a “lack of motivation to coordinate by relevant sectoral bodies, because the transaction costs of coordination are perceived higher than the benefits.”⁷ According to Perrone *et al.*, “it is rare that nexus boundaries align with traditional management units or administrative boundaries – for instance, river basins,

urban areas, or even geopolitical divisions.”⁸ Cairns and Krzy-woszynska found that within natural resource conversations in the United Kingdom, the comprehension of the term *WEF nexus* is “plural, fragmented, and ambiguous.”⁵

Another further complication lies in the inability of the nexus to cohesively move from theory to practice. Weitz *et al.* states that “although the nexus literature identifies some barriers to achieving coherence it does not clearly explain why the barriers are present, what influences them, and how they can be acted upon. These gaps disconnect the nexus literature from the governance processes it ultimately seeks to influence.”⁴ *The Guardian* notes that “under these conditions, it is difficult to see how an integrated solution could emerge that is significantly different to what each sector by itself would pursue.”⁹ Although the WEF nexus covers a complex and intricate set of relationships spanning multiple sectors, the inherent gaps in its application inhibit meaningful change in the decision-making and policy-making processes.

By drawing on an extensive review of research and case studies, this paper evaluates the effectiveness of the WEF nexus with a focus on governance and implementation. This paper also aims to enhance our understanding of the impact local decentralized projects can have on the water and food dimensions of the nexus. A few examples of the local decentralized projects discussed in the paper are: improved cook stoves in Ecuador; community-based jatropha in Bati Woreda, Ethiopia; and biomass gasifier-based mini grid in Garkha Village, Bihar, India (Table 1). Finally, this paper suggests that integrating livelihoods with the WEF nexus approach is the key to achieving sustainable development. If the status quo is maintained, it will be impossible to meet the concurrently huge demands in water, energy, and food in the next decades. The importance of water, energy, and food in sustainable development is highlighted by the fact that they each have their own sustainable development goal; however, their significance extends to economic development, governance, and environmental systems.

■ Discussion

Based on a review of the nexus literature, Weitz *et al.* identified three governance gaps that render the nexus concept disconnected from the decision-making and policy making processes it ultimately seeks to influence.⁴ One common thread that emerges in the literature is that policies are fragmented across the water, food, and energy sectors. Varying explanations are provided explaining why fragmented policies pose a problem. Three perspectives emerge while discussing governance issues related to the WEF nexus. The first perspective is based on the idea that failing to account for connections between sectors could worsen resource scarcity and induce conflicts. The second perspective relates to economic rationality. Accordingly, the nexus is seen to improve policy cost-effectiveness and resource-use efficiency. This economic rationality perspective encourages green economic growth. However, policy coherence is undermined by a lack of communication and clarity on the rights and responsibilities across sectors. The third perspective focuses on equity and social progress. With this perspective, the key barriers to policy coherence are unequal

perspective, the key barriers to policy coherence are unequal distribution of power, voice, access to information, resources, and capability among actors. Although there is an agreement that cross-sector collaboration and coordination are desirable, little is explained on how to enable coordination across sectors

Challenges in the governance of WEF nexus:

According to Bhaduri *et al.*, governing the nexus involves dealing with the plurality and interaction of policies between the different sectors.¹⁰ Despite the evidence of strong connections between the water, energy, and food sectors as well as the attention the nexus has received from development agencies, policy analysts, and other stakeholders, the “silo mentality” still exists. This means that food, energy, and water challenges continue to be addressed within sectoral boundaries in terms of projects and policy decisions.¹ Formulating and addressing policies in silos does not guarantee water, food, and energy security and sustainability. For example, governments often design agricultural policies and subsidy programs for fertilizers without considering their interconnectedness with the energy and water sectors. On a similar note, governments provide energy subsidies to farmers without taking into account the implication for groundwater depletion.

For nexus governance to be successful, the values of different stakeholders and interest groups have to be taken into account in a fair and transparent manner. Additionally, the private sector, bureaucratic structures, and informal networks need to be effectively combined. Bhattacharyya *et al.* discussed the implications of the WEF nexus in the 15 countries that make up the Economic Community of West African States (ECOWAS).¹¹ These countries have a combined population above 300 million and face the prospect of doubling their population by 2030. Almost 58% of the population lives in rural areas and 42% are below the poverty line. While the WEF nexus has inspired the member countries, the degree of success varies. The sector-based approach to policy making continues and the implementation of an integrated approach is rare. The centralized top-down approach of governance prevails while the decentralized bottom-up mobilization of initiatives is lacking. Even when the decentralization has been promoted, the delegation of appropriate authorities has not taken place in ECOWAS.

Nexus governance requires political support for successful implementation at the local level.¹⁰ India is the world’s single largest user of groundwater with 19.8 million agricultural wells and tube wells.^{12,13} While farmers in India have always used groundwater for irrigation, its rise as the main source of irrigation coincided with the Green Revolution of the 1970s. Many states in the northern and western parts of India face extreme groundwater exploitation. In response, policies were designed to prevent excessive extraction of groundwater through interventions such as the West Bengali policy of groundwater pricing based on metered usage rather than a flat rate.¹² This policy has been successful in reducing groundwater usage and degradation. In northern and western states, electricity feeders for agricultural uses have been separated from feeders for domestic and industrial electricity use, thus controlling over-extraction of groundwater.¹²

Policies made at the national level can have a significant impact on local communities. For example, dam construction significantly impacts water access for local stakeholders. When hydropower development takes place in transboundary river basins, cross-sectoral challenges and policy options are inter-linked at different levels.¹⁰ Hensengerth analyzed the limited influence an organization centered on water issues has on hydropower development and addressed the issue of authority in international development and hydropower policy using the Xayaburi Dam in the Lower Mekong Basin as a case study.¹⁴ The Xayaburi Dam elevates WEF nexus issues to the trans-boundary level. Seasonal differences in water release and use requirements for irrigation and energy production have caused conflicts with significant negative impacts on the local communities. There is a need to protect the water use rights of local stakeholders.¹⁰

Mayor *et al.* proposed an assessment methodology for WEF nexus implementation at the basin level and applied the tool to a case study of the Duero River basin in Spain.¹⁵ The case study illustrates how to understand trade-offs and synergies, diagnose the level of political coordination needed, and identify existing and potential solutions to improve water, energy, and food resource management in the region.¹⁵ Some of the most important issues found were the limitations posed by rising energy prices for irrigated agriculture due to modernization, limits to treating water, and the emergence of new energy-related water demands in hydraulic fracturing for oil and gas. Mayor *et al.* found that freshwater is being increasingly consumed for oil and gas exploration, often in water-stressed areas. It was suggested that co-optimization of resources is essential to meet energy demands without depleting remaining freshwater reserves.¹⁵

According to Mayor *et al.*, “the WEF nexus can be considered as a framework that helps connect, coordinate and reinforce individual water, energy and food management policies by paying special attention to the identification, understanding, and characterization of interconnections and tradeoffs, in order to build aligned and coherent strategies/actions that permit synergies. This should be applicable both within the political sphere, at either national, regional or local scales, and at a business or even household scale, where resource management and optimization is also required.”¹⁵ It is clear that there are gaps in the WEF nexus implementation. Due to its multi-sectoral approach, the nexus becomes difficult to translate into policy. For the WEF nexus to be applicable to communities of different scales, regions, and environments, a significant effort must be placed on its translation into policy.

The risks and uncertainties related to climate change further complicate nexus governance. Therefore, it is important to clearly understand the linkages and vulnerabilities of these resources. Interdisciplinary research is necessary to assess the effect of climate change on water, energy, food, and their related ecological processes.¹⁶ Without research, it is difficult to evaluate the WEF interactions and quantify the benefits and costs between the sectors.¹⁶

Assessment tools for WEF nexus implementation:

Several scholars have discussed assessment tools that can guide nexus implementation at different scales. For example, the multi-scale integrated analysis of societal and ecosystem metabolism (MuSIASEM) is a framework that focuses on a multilayer analysis of water, food, and energy and takes into account labor, the hours of human activity, and all other elements that could change during analysis. The climate, land, energy, and water systems (CLEWS) framework applies to a number of studies and provides a network for looking at interlinkages while also including stakeholder engagement in testing. The CLEWS model is broken down into three sub-models each focused on a different aspect of water, food, or energy that is later centralized within the larger framework. A number of digital models also exist in mapping out the nexus. The Commonwealth Scientific and Industrial Research Organization's national outlook model (NOM) was created in Australia and provided input access for data later translated to a national scale. However, the NOM framework is very data-heavy and costly, meaning that the implementation of it is largely restricted to Australia. Another model is the WEF Nexus Tool 2.0, which distinguishes itself by allowing for user input data and scenarios to develop a cleaner visualization of nexus goals. This model was used in Qatar to map agricultural crop production but cannot address wider concerns of livestock or processed food.¹⁷ Finally, the Foreseer tool has been used in the United Kingdom and generates scenarios by taking into account natural resource supply. The tool has been able to link models together in developing a final service for the area being targeted. However, no efforts have been made to address more local levels, and this may prove challenging due to limited data.

There have been several solution-based contributions to scaling the nexus with one of the largest being to take political systems and stakeholders into account rather than just technical variables. Another is to build and encourage the establishment of inclusive institutions with the capacity to take on policy-building via the nexus approach, with both horizontal and vertical coordination. In regard to knowledge-based changes, a major concern of researchers is the lack of open data on the use of the nexus.

Capacity building and knowledge synthesis for sustainable implementation of the WEF nexus:

Capacity is best strengthened by developing coordination mechanisms for existing institutions to support addressing WEF nexus objectives. Strong coordination mechanisms allow synergies to develop between the three sectors. A variety of institutions at local, regional and national levels, including the private sector, international development partners, and civil society organizations can jointly meet capacity development needs to not only improve the understanding of interlinkages but also generate greater efficiencies through joint management and operations, as well as increased opportunities of inter-institutional innovations.¹⁰ Individual capacities do not develop in a vacuum but are embedded in wider institutional networks, which adds to the complexity of capacities. Emerging evidence shows that capacity develop

ment can be effective from the individual to organizational levels but can take time to achieve outcomes.¹⁰

Along with strengthening capacity for existing institutions to address the nexus objective and face nexus challenges, the other challenge is a lack of synthesis for nexus knowledge. There is a lack of communication among the institutions that collect data and the decision makers who use these data. Currently, most data-providing portals are scattered, and unconnected. There is also a gap between data required by decision makers and easily accessible data. Resultingly, there is a need to build comprehensive datasets by integrating data from different portals. To increase capacities for analyzing such integrative datasets, the next generation of scientists and practitioners needs to be trained in the interconnectedness of water, energy, and food. Organizations will need to work across administrative boundaries to turn nexus thinking into meaningful practices.

Decentralized energy projects and the WEF nexus:

Guta *et al.* employed a qualitative case study methodology to study the complex interactions between the WEF nexus and energy transitions within six local contexts in developing countries.¹⁸ The selected locations represent different geographic, socioeconomic, and institutional contexts from India, Ethiopia, China, Ecuador, and Nepal.¹⁸ The case studies incorporated four renewable energy sources – biomass, hydro, solar, and wind – and different experiences of successes and failures. The China case study focused on the household biogas project in the Chunfeng village on the Sichuan–Yunnan province border. Biogas usage mitigated the contamination of drinking water and reduced the pollution of local surface water and groundwater caused by chemical fertilizers. Additionally, use of biogas instead of firewood helped avoid damage to watersheds and forests. There were some barriers to implementation, including overemphasis on low cost, which resulted in the short life of the digesters and stoves. Another case study focused on the improved cook stoves in the highland region of Ecuador.¹⁸ Improved cook stoves resulted in reduced wood consumption for cooking, which reduced deforestation. Some barriers to implementing the improved cook stoves were related to strong bonds to traditional cooking practices involving wood.

In the Ethiopian case study, the jatropha plant was substituted for biomass and fuel. Using jatropha reduced land degradation and improved soil and water retention. Barriers to the implementation were related to low jatropha yields, poor market linkages, and a lack of financial and technical support. In another case study, a biomass gasification plant was installed in a village in Bihar, India to overcome the problem of unreliable electricity supply from the grid. The plant provided reliable power to ten water pumps in the village at a reasonable price. This positively impacted agricultural production; farmers were able to remove water from the waterlogged field and use it for farm production. Although the concept of biomass gasification has gained popularity over the last decade, there are several barriers, such as the unwillingness of banks to finance such technologies.

The last case study, a micro hydro plant in Siklesh Village in Nepal provides access to electricity for a variety of domestic and productive uses including operation of agricultural processing mills. The availability of electricity has benefits in terms of food preparation, thanks to higher quality lighting in comparison to kerosene lanterns. Even though switching from kerosene to electricity for lighting has been clearly observed, the generated electricity has not been able to replace fuelwood for cooking. All the case studies by Guta *et al.* indicate that alternative energy sources positively contributed to households and livelihoods. The case studies identified barriers mostly related to installation of technologies, cost of maintenance, and operations.¹⁸

Integrating local livelihoods and the WEF nexus:

Biggs *et al.* explains that the WEF nexus was initially framed from a resource security perspective to protect against risks to resource availability.¹⁹ However, framing the WEF nexus in terms of resource security instead of distributive justice shifted the focus from the needs of the poor toward global issues, large infrastructure developments, and macro resource flows. The focus on the challenges faced by the poor at local, community, and household levels is missing. The local livelihood aspect of the nexus has not been part of the nexus research.²⁰ Billions of people who lack access to safe drinking water and energy services often depend on agriculture for their livelihoods. The current siloed sectoral approach directly impacts livelihoods as the impoverished population generally lack the resources to adapt to change.

A sustainable livelihood is defined as “capabilities, assets, and activities required for a means of living. It is deemed sustainable when it can cope with and recover from stresses and shocks and maintain or enhance its capabilities, assets, and activities both now and in the future, while not undermining the natural resource base.”²¹ Transitioning the WEF nexus to include local households and livelihoods has been a challenge and an ongoing topic of discussion and research; the nexus was originally created to be implemented using an implicit top-down approach that often reiterates power dynamics and disregards local concerns.

In a survey of 549 households in Ethiopia, locals were asked about the operation of two large irrigation and hydro-powered dams using the nexus model. The study found that the effects of water infrastructure did not necessarily translate to livelihoods; the local people perceive limited access to both irrigation and electric power. According to the study, “assessing the nexus at household level revealed the concerns of local communities whose views and concerns are obscured in national- and global-level debates. Large-scale water infrastructures are characterized by uneven distribution of costs and benefits.”²⁶ The WEF nexus must go beyond and examine sociopolitical benefits to populations where needs must be met. The question of whose needs are being met and who is being undermined are still unanswered.

To understand local community perceptions of the WEF nexus challenges, Wolde *et al.* conducted a survey of local farmers and representatives of the Gidabo sub-basin in central

Ethiopia.²² They found that the majority of people are susceptible to recurrent drought, food insecurity, and extreme poverty. Lake Abaya, the main water source, shows signs of overuse and extraction. The local community perceives natural resources as sources of income and subsistence which further aggravates natural resource degradation.²² Therefore, many scholars suggest that local environmental conservation should be an important component of the WEF nexus. More research is needed to understand the local community perception of their livelihoods and WEF nexus resources.

The nexus is crucial for rural household communities but has not been thoroughly researched for those areas. Despite expectations of the nexus to support the *United Nations's* Sustainable Development Goals, the poor have neither been targets nor beneficiaries of the nexus.²³ Additionally, very few authors have developed step-by-step frameworks for putting the nexus into practice.

Terrapon-Pfaff *et al.* created a thorough framework of linkages for operationalizing the nexus using a household model.¹ Their method was to “analyze the linkages between small-scale energy projects in developing countries and the food and water aspects of development.”¹ Their paper suggests a four-step nexus assessment approach to operationalize the WEF nexus at the local level: (1) qualitatively map the WEF nexus links, (2) quantify the links, (3) identify critical links, and (4) leverage the results to improve project design and implementation.¹ While this is a good start in mapping out local implementation of the nexus, more emphasis needs to be placed on the specificities of integration of water and energy into local-level planning.

Another important aspect to consider within the realm of the nexus intersecting with social science is the data accountability based on location. In a case study of the WEF nexus in Kenya, researchers came across the issues of organization and coordination in the local policy sector. Kenya is a water-scarce country, with 80% of the country being arid or semi-arid. Kenya's legislative institutions are divided in a way that created a lack of communication between sectoral departments. At the county level, there was a clear inability to follow the nexus due to a lack of capacity and reluctance of the central government to pass responsibility down to local counties.²⁴

A similar study was conducted in Indonesia, a country with one of the highest rates of deforestation in the world. Unlike Kenya, the nexus was able to be implemented at the district level. However, the structure of the government system was not able to maintain the nexus; specifically, there was no provincial water management system implemented in the country.⁶ These two studies emphasize the fact that regardless of where the nexus is being used, the decisions of the nexus are political. The WEF nexus approach combines the trade-offs between its key resources and the competing demands of the resources for human and economic development.²⁵ The rising issues of resource scarcity and climate change increases the significance of such trade-offs. There must also be more thought placed into strengthening local governments, especially in rural areas, so they can handle large government institutions.

A growing body of research has focused the value of nexus-based approaches for evaluating the effects of development on livelihoods and for promoting sustainable livelihood practices.¹⁹ For example, Rasul's analysis of the Hindu Kush Himalayan region from a nexus perspective has highlighted the positive effects that stronger regional integration across the WEF sectors has on livelihoods.²⁶ Bouapao has taken the WEF nexus approach further by modeling the cumulative effect of impending development decisions designed to improve food, water, and/or energy security on livelihoods.²⁷ Population in the lower Mekong region could experience changes in household food and income levels as a result of development decisions that impact fish, crops, vegetables, wetlands, and non-timber forest products.²⁷ In Myanmar, foreign investment to intensify production from the agricultural and energy sectors may have harmful effects on rural populations who rely on shared land and water resources but have insecure access.²⁸ Examples in Nicaragua demonstrate how sustainable rural livelihoods can be promoted in harmony with the nexus framework, such as rainwater-harvesting systems for small landholders using small-scale water capture and storage systems. These provide potential for increasing the consumption of underutilized but abundant food and flour as well as producing bioenergy from sugarcane.²⁷

Environmental livelihood security: The way forward:

Environmental livelihood security (ELS) refers to the challenges of maintaining global food security and universal access to fresh water and energy to sustain lives and promote inclusive economic growth while sustaining key environmental systems.¹⁹ The term was theorized to address a lack of consideration of livelihoods within nexus frameworks which is required to ensure that WEF securities enable sustainable development and livelihoods. A system's ELS is met when the human demand on the environment and environmental impacts on humans are balanced. The desire to achieve sustainable systems places the concepts of sustainable development and sustainable livelihoods as the core ideas of ELS.

The ELS framework acknowledges the mutually dependent relationship between water and livelihoods: (i) water is needed to support livelihood activities such as fisheries or irrigated agriculture; and (ii) livelihood activities and capitals may contribute to the preservation (or depletion) of water supplies and access. For example, physical capital (infrastructure) may enable more efficient water extraction and transportation and financial capital (public or private funds) may assist in implementing more sustainable practices in water use or purchasing alternative supplies.¹⁸

■ Conclusion

The WEF nexus was created to examine the synergies and trade-offs between these three key sectors in different communities. Through reviewing the current literature as it applies to the nexus governance and implementation, there is a clear need for a social science and governance lens rather than a technical perspective. If concrete changes are to be made, governments will need to integrate the nexus into policy decisions. Despite the nexus's scientific ingenuity, significant time must

be spent in analyzing how the nexus will account for policy and individual livelihoods. If the nexus is to be scaled down from a national perspective to more regional areas, there must be more data collected to ensure that proper policies are implemented through the nexus's findings.

Table 1: Summary of findings from selected past research.

Paper	Findings
Bhaduri, A., Ringler, C., Dombrowski, I., Mohtar, R., Scheumann, W. (2015) ⁹	A broad involvement of stakeholders to work collaboratively towards sustainable development is an important step for successful implementation of WEF nexus
Terrapon-Pfaff, J., Ortiz, W., Dienst, C., Gröne, M. C. (2018) ¹	Complex links exist between sustainable energy projects and the food and water sectors. The energy needs addressed are often directly connected to food or agricultural activities or are connected indirectly by providing energy for water use in agriculture. A more systematic approach integrating the water and food pillars in energy planning at local level in the global south should be advocated to avoid trade-offs and enhance the development potential of energy projects
Cairns, R., Krzywoszynska, A. (2016) ⁵	The mobilization of the nexus terminology can best be understood as symptomatic of broader global science policy trends, including an increasing emphasis on integration as an ideal; an emphasis on technical solutions to environmental problems; achievement of efficiency gains and 'win-wins'; and a preference for technocratic forms of environmental management
Guta, D. D., Jara, J., Adhikari, N. P., Chen, Q., Gaur, V., Mirzabaev, A. (2017) ¹⁸	The decentralized energy projects have positively contributed to household and community livelihoods and are an integral component of the WEF Nexus.
Wolde, Z., Wei, W., Kumpeng, W., Ketema, H. (2020) ²²	The WEF nexus resources have the potential to contribute to different livelihood aspects (i.e., human, physical, social, financial, natural, and environmental).

There are billions of people in rural areas who depend on agriculture for their livelihoods. Governance mechanisms should be strengthened at local levels to improve well-being and build resilience into rural livelihoods. Embracing a cross-sector approach will reduce the negative impacts of policies on each individual sector. Policies need to become more inclusive and better coordinated with each other. Innovative research and technologies are important to support the implementation of the nexus at local levels. The initiation and implementation of decentralized energy projects that recognize the interconnectivity of the water, energy, and food sectors are great steps in this direction. These decentralized energy projects use resource-efficient technologies that enhance sustainability at the local level, but more are needed to better support land, water, and energy. The use of green energy sources such as wind and solar can not only increase energy availability and access, but also easily reach the poor rural areas.

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Exploring Human Longevity: The Impact of Climate on Life Expectancy

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ABSTRACT: This research aims to investigate whether climate has an impact on life expectancy. In analyzing economic data from 172 countries that are publicly available from the United Nations World Economic Situation and Prospects 2019, as well as classifying all countries from different regions into hot or cold climate categories, the authors were able to single out income, education, sanitation, healthcare, ethnicity, and diet as constant factors to objectively quantify life expectancy. By measuring life expectancies as indicated by the climate, a comprehensible correlation can be built of whether the climate plays a vital role in prolonging human life expectancy and which type of climate would best support human life. Information gathered and analyzed from examination focused on the contention that human life expectancy can be increased living in colder regions. According to the research, an individual is likely to live an extra 2.2163 years in colder regions solely based on the country's income status and climate, while completely ruling out genetics.

KEYWORDS: Earth and Environmental Sciences; Life expectancy; Climate Science; Longevity; Income groups.

■ Introduction

To better understand the study, it is crucial to understand the difference between life span, life expectancy, and longevity. According to the United Nations Population Division, life expectancy at birth is defined as “the average number of years that a newborn could expect to live if he or she were to pass through life subject to the age-specific mortality rates of a given period.”¹ When addressing the life expectancy of a country, it refers to the mean life span of the populace in that country. This factual normal is determined dependent on a populace in general, including the individuals who die during labor, soon after labor, during puberty or adulthood, the individuals who die in war, and the individuals who live well into mature age. On the other hand, according to News Medical Life Sciences, life span refers to “the maximum number of years that a person can expect to live based on the greatest number of years anyone from the same data set has lived.”² Taking humans as the model, the oldest recorded age attained by any living individual is 122 years, thereby implicating that human beings have a lifespan of at least 122 years. Life span is also known as longevity.

As life expectancy has been extended, factors that affect it have been substantially debated. Consensus on factors that influence life expectancy include gender, ethnicity, pollution, climate change, literacy rate, healthcare access, and income level. Other changeable lifestyle factors also have an impact on life expectancy, including but not limited to, exercise, alcohol, smoking and diet. Nevertheless, life expectancy has for the most part continuously increased over time.

The authors' study aims to quantify and study the factors that affect human life expectancy. According to the American Journal of Physical Anthropology, Neolithic and Bronze Age data collected suggests life expectancy was an average of 36 years for both men and women.³ Hunter-gatherers had a higher life expectancy than farmers as agriculture was not common yet and

people would resort to hunting and foraging food for survival. From then, life expectancy has been shown to be an upward trend, with most studies suggesting that by the late medieval English era, life expectancy of an aristocrat could be as much as 64 years; a figure that closely resembles the life expectancy of many populations around the world today. The increase in life expectancy is attributed to the advancements made in sanitation, education, and lodging during the nineteenth and mid-twentieth centuries, causing a consistent decrease in early and midlife mortality. Additionally, great progress made in numerous regions of well-being and health, such as the discovery of antibiotics, the green revolution that increased agricultural production, the enhancement of maternal and child survival, and mortality from infectious diseases, particularly human immunodeficiency virus (HIV)/ AIDS, tuberculosis (TB), malaria, and neglected tropical diseases (NTDs), has declined. According to the World Health Organization (WHO), global average life expectancy has increased by 5.5 years between 2000 and 2016, which has been notably the fastest increase since the 1950s.⁴ As per the United Nations World Population Prospects, life expectancy will continue to display an upward trend in all regions of the world. However, the average life expectancy isn't predicted to grow exponentially as it has these past few decades. Projected increases in life expectancy in Northern America, Europe and Latin American and the Caribbean are expected to become more gradual and stagnant, while projections for Africa continue at a much higher rate compared to the rest of the world. Asia is expected to match the global average by the year 2050. Differences in life expectancy across regions of the world are estimated to persist even into the future due to the differences in group incomes, however, income disparity between regions is forecasted to diminish significantly by 2050 (Figure 1).⁵

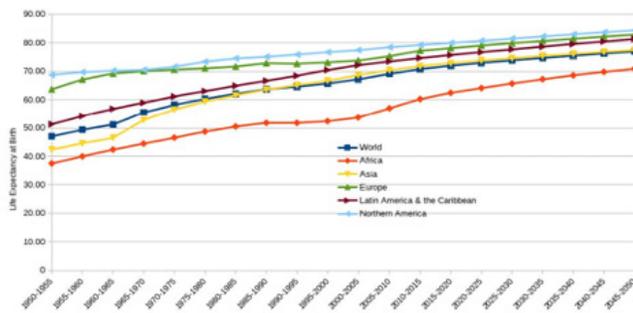


Figure 1: Trend line of life expectancy from 1950 to 2050 by world regions. Source: Life Expectancy by Region from UN World Population Prospects. The graph illustrates exact increases in life expectancy until 2017, and then predicts trends to 2050.

The blue zone areas (Figure 2) are five regions across the world, identified as ‘the hot spots of longevity’ according to National Geographic, where people live much longer than average.⁶ All of these cities house a population where many individuals hit the 100 years milestone. When the authors encountered “blue zone” areas in multiple studies about factors affecting human longevity, the authors observed a pattern in the placement of these blue zone areas on the world map—they are all located from 1100 to 4500 km north of the equator, and all experience Mediterranean or tropical climates, or what the authors classified as ‘warm’ in accordance with the designed classification system. The factors attributed to the longer life spans of these communities are healthy diet, daily exercise and adopting a low-stress lifestyle. However, in no study has climate ever been mentioned as a contributing factor to extending life span in these blue zone areas, when they so clearly have warm and coastal climates as a common factor. This got the authors thinking: does climate play a role in or affect human life expectancy? Scientists have debated the role of climate and temperature in human longevity for decades. On first glance, and while considering blue zone areas, it could be suggested that humans live longer on average in warmer climates. This side of the argument is supported by multiple studies, such as one by Professor F. Ellis of Yale University, who found that deaths in the United States increased by 13% in the winter, compared to the summer, using data from the 1950s.⁷ The opposite side of the debate claims that living in colder climates slows down ageing and thus extends life expectancy. The principal theory corroborating this side of the debate is the ‘free radical theory’ that states organisms age because of buildup in cells and damage from metabolic activities in particular, a byproduct under the name reactive oxidative species (ROS). The theory claims that exposure to colder temperatures slows down the rate of ageing—thus slowing the release of the byproduct ROS so that less cellular damage occurs over time and thereby slowing ageing and increasing life expectancy.⁸

The authors’ aim with this study was to investigate how rising and warmer temperatures will affect human life expectancy and longevity, which is where climate change comes into play. According to the International Actuarial Association, climate change has already demonstrated that it will have critical and extensive impacts around the globe.¹⁰ The most significant

objective for this paper was the mortality of the populace. Climate change is enduring danger to our planet. Largely, this will result from critical rises in CO₂ and methane levels in the Earth’s atmosphere, environment, and oceans that have been amassing through many decades. This risk will unfavorably influence our water, air, climate, and ecosystems, bringing about numerous changes to our environment, including augmentations in temperature, changes in precipitation, for example, heavy rains and longer-enduring droughts, a rise of infrequent extreme weather, as well as sea and ocean levels. A definitive impact of these climatic changes will influence human wellbeing, life, and property. Be that as it may, the degree of climate change remains a present-day and grave future dilemma.



Figure 2: Cities that form the ‘blue zone’ areas on the world map.⁹

Acknowledging the arguments from both sides of the debate, and having read, analyzed and reviewed the literature available on this topic, the authors set out to quantify any impacts climate has on longevity. By quantifying life expectancy according to climate, a clear correlation can be established of whether climate plays an integral role in human longevity and which type of climate best supports human life. Data collected and examined from the research points towards the argument that human life expectancy can be extended by living in colder climates. Colder climates overwhelmingly display higher life expectancy in all four socio-economic country classifications.

■ Methods

The countries of the world were classified initially based on four income groups: high income, upper-middle-income, lower-middle-income, and low income. These classifications were taken directly from the United Nations World Economic Situation and Prospects 2019 Country Classification.¹² Following this, the authors further classified the countries in each income group as ‘mainly warm’ and ‘mainly cold’ (Figure 3). This closed question approach was critical to establish a correlational link between climates, temperature, and human life expectancy. Countries with mainly tropical, subtropical, sub-Saharan, Mediterranean, savanna, and equatorial climates were classified under warm. These countries have dry, arid, hot, and humid temperatures with rainy winters and saw almost no winters with freezing temperatures. Countries with more seasonal climates, temperate, continental, oceanic, maritime, and

highland climates were grouped under cold. Classifying countries based on income groups was essential to keep climate a constant factor as far as possible because income directly affects access to education, sanitation, access to healthcare and diet- all of which also greatly affect human life expectancy.

For countries such as the United States, China, and France that experience different climates in different regions or states, the authors collected data about individual state/provincial climates and designated the titles 'mainly warm' or 'mainly cold' according to the mode or majority. For example, the United States comprises 50 states, out of which 29 experience marine, cold, or very cold climates and 21 experience mixed-dry and hot-humid climates. As a result of this majority, the authors classified the United States as 'mainly cold'.

Figure 3 was drawn up using the Microsoft Excel software based on the number of regions around the world that were classified as warm and cold. Thereafter, mean life expectancies, standard deviation, minimum, and maximum values for each country were calculated based on the data made available from the United Nations World Mortality Report 2019.¹¹ Tables 1 and 2 provide summaries for this data while Figure 6 paints a graphical representation.

To create Figure 5, the authors divided the world into twenty-two regions, irrespective of income groups. This was done to single out genetic factors and ethnicity, which too influence life expectancy. The twenty-two regions were then divided into warm and cold regions. The authors classified fifteen as warm and seven as cold. Their mean ages were then drawn up, once again using the United Nations World Mortality Report 2019.¹¹



Figure 3: World map classified as warm and cold according to the authors' designed system. Key: warm is represented by yellow, cold by green and data not available as pink.

To calculate values for ANOVA, the authors used spreadsheet software Microsoft Excel to calculate the $(X-\text{mean})^2$ value of each observation. The observations were mean life expectancies of each country listed on the United Nations World Mortality Report 2019.¹¹ These observations were then divided into eight groups: warm socio-economic groups and cold socio-economic groups in accordance with the system described above. The sum of $(X-\text{mean})^2$ was calculated for each

group. Using this data, variance within each group, total sum of variance, variance within each group, degrees of freedom, F-value, and p-value were also calculated correspondingly using the formulas shown in Figure 4. The calculation was that standard to a typical ANOVA test.

■ Results and Discussion

total sum of variance = sum of $(X-\text{mean})^2$ of all observations

variance within each group = sum of $(X-\text{mean})^2$ of each group

variance between each group = total sum of variance - variance within each group

degrees of freedom (numerator) = no. of groups - 1

degrees of freedom (denominator) = no. of observations - no. of groups

F-value = (variance with each group/degrees of freedom denominator)/variance between each group/degrees of freedom numerator

p-value (using a spreadsheet program) = *fdist*(F-value, numerator degrees of freedom, denominator degrees of freedom)

**X denotes an observation*

Figure 4: Formulas used while calculating ANOVA.

With global warming and a continuous increase in greenhouse gases over the decades, climate change has become a peril to mankind. Taking this as a motivation, the research report aims to find a clear link to rise in temperature and its direct effects on human life expectancy and longevity. The question of whether climate plays a quantifiable role in human longevity and life expectancy was tested by first classifying all countries according to income groups. Within these income groups, the countries were then further classified as 'mainly cold' and 'mainly warm' in order to mathematically measure the effects of climate and temperature. The means of ages and life expectancy were then calculated for each income group and cold and warm climates within those groups.

The pie chart is a representation of all the different regions around the world classified as 'mainly cold' or 'mainly warm'. The intent was to show the temperature distribution around the globe - with 33.8% being mainly cold regions and 66.2% being mainly warm regions (Figure 5).

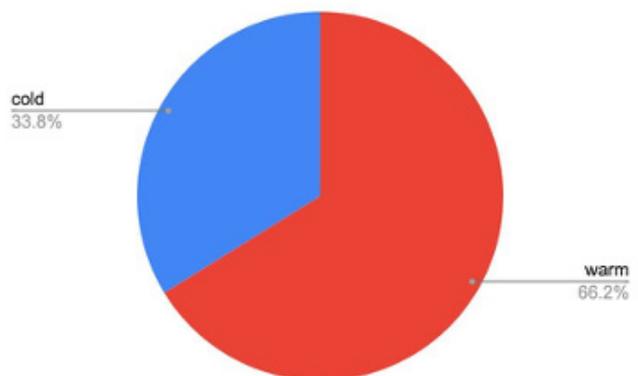


Figure 5: World region climates classified as percentages.

Across all income groups, the mean life expectancy was higher in the colder climates. With the lowest difference being between the lower-middle income groups, where colder climates displayed an average life expectancy that was 0.2371658 years higher than their warmer climate counterparts. The greatest difference was observed between the low-income groups where the average life expectancy differed by a large

5.9098901 years in the colder climates compared to warmer ones. On average, the minimum life expectancy was higher in all income groups for the colder climate classification, except for the lower-middle-income group where they were almost equal. Additionally, the standard deviation for all income groups in the warmer climates was more spread out than the colder climates, suggesting that the warmer climate regions across all income groups saw a greater disparity in the ranges of life expectancy (Table 1, Table 2, Figure 6).

ANOVA, or analysis of variance, is a testing method to prove the statistical significance of the data collected. The main idea behind ANOVA is to determine how much of the variance comes between groups and how much comes from within groups. According to the calculated ANOVA test, the variance between each group (refer to Table 1 for grouped data) was more than double the variation within each group, which gives the authors a suggestion that climate does have a significant effect on life expectancy. To further test this, the authors calculated the F-value and p-value. The F-value of 49.88126711 is much larger than 1.0, while the p-value comes to exactly 0.00 and is less than 0.05. The p-value denotes that the probability of the null hypothesis taking place is 0% and the large F-value means that variation within each group is more than what could be expected of chance. Together these values indicate strong evidence against the null hypothesis (climate doesn't affect human life expectancy) and accepts the alternative hypothesis (climate does play a role in human life expectancy), while proving the results statistically significant (Table 3).

Table 1: Summarized statistics of data for warm climate longevity. Data Sources for life expectancy: United Nations World Mortality Report 2019.¹¹

Warm Climate Longevity	mean	std.dev	min	max
high income	79.4	3.372120994	73.5	83.6
upper-middle income	73.68648649	4.876540136	58.7	80.3
lower-middle income	68.82647059	5.722645543	54.7	77
low income	62.16153846	4.460410467	53.3	66.6

Table 2: Summarized statistics of data for cold climate longevity. Data Sources for life expectancy: United Nations World Mortality Report 2019.¹¹

Cold Climate Longevity	mean	std.dev	min	max
high income	80.91481481	2.412579804	75.3	84.6
upper-middle income	74.89	1.418489024	73	76.9
lower-middle income	69.06363636	5.60754362	54.3	73.8
low income	68.07142857	3.881457753	61.5	72.3

Table 3: Data calculated for ANOVA.

Total Sum of Variance	10055.85444
Variance within each group	3292.753773
Variance between each group	6763.100667
Degrees of Freedom:	
numerator	7
denominator	170
F-value	49.88126711
p-value	0

droughts, flooding, extreme weather conditions, more infectious diseases spread by vectors, also possibly genetic changes

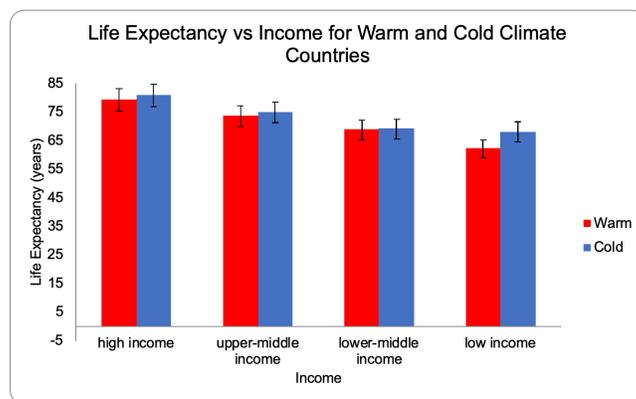


Figure 6: Tables 1 and 2 data represented graphically to show mean ages and data variation according to various income groups. Data Sources for income classification: United Nations World Economic Situation and Prospects 2019 Country Classification.¹²

The bar chart represents the mean life expectancy across 22 regions around the world that are further classified as 'mainly warm' (red) which comprises of 15 regions, and 'mainly cold' (blue) which accounts for the remaining 7. Upon examination, the highest life expectancy was found in Southern Europe (82.3 years), Western Europe (81.9 years) and Northern Europe (81.2 years). Within these three regions topping the list of life expectancy, two are classified as mainly cold. On the other hand, three mainly warm regions: Western Africa (57.9), Middle Africa (59.9) and Southern Africa (63.8) have the lowest life expectancies in the world (Figure 5).

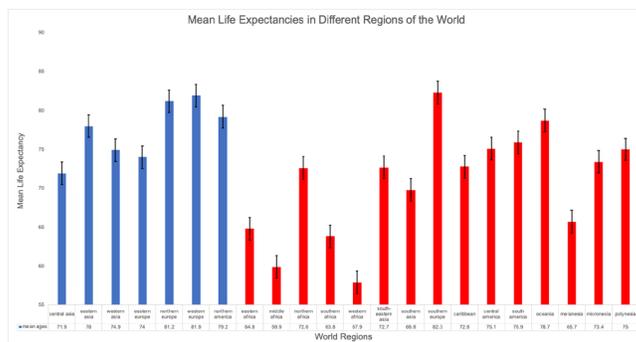


Figure 7: Mean life expectancies in different regions of the world. Blue bars illustrate colder regions, while red bars denote warmer regions.

Data collected and examined from the research points towards the argument that human life expectancy can be extended by living in colder climates. Colder climates overwhelmingly display higher life expectancy in all four of the socio-economic country classifications.

All the data collected, recorded, and calculated showed average life expectancy to be higher in colder climates. Whether that difference was just 0.2371658 years higher in the case of lower-middle-income countries, or 5.9098901 years higher in the case of low income; the results indicate that living in colder climates can extend longevity. What can be concluded from this data is that climate change will have definite and lasting impacts on human life expectancy and lifespan. Rising and warmer temperatures will bring a lot of other complications to the table, including but not limited to famines,

that could be triggered by these increasing temperatures - effects that no one knows or haven't yet been predicted.

According to the National Geographic, "Since 1906, the global average surface temperature has increased by more than 1.6 degrees Fahrenheit (0.9 degrees Celsius)". Scientists around the globe have already documented many drastic changes in the environment due to climate changes. Glaciers have contracted, ice on waterways and lakes is collapsing earlier, there have been peak rises in sea levels, intense and longer heat waves, plant and animal ranges have moved with many migrations noticed, and trees are blossoming sooner.¹³ Moreover, the World Health Organization (WHO) states that air pollution contributed by the emissions of fossil fuels as a result of the greenhouse effect, particularly sulfur and nitrogen-oxides can lead to respiratory diseases like asthma which can be seen in adults and children. It is stated that an estimated 7 million people die worldwide annually from air pollution.¹⁴

It is essential to stop climate change as it not only dramatically causes natural habitats to become isolated remnants, raises in sea levels, and increases extreme weather, but it also has a negative impact on human life and prosperity. For instance, the WHO has stated, "natural hazards like heat waves can burden health and emergency services and also increase strain on water, energy and transportation resulting in power shortages or even blackouts. Food and livelihood security may also be strained if people lose their crops or livestock due to extreme heat".¹⁵ Agriculture is one of the areas most affected, where farmers are bound to face attacks from weeds and pests which influence yield. Additionally, certain extreme events undermine crop yields. A study at the Bryan College of Health Science shows that the same CO₂ amassing in our environment due to fossil fuels is altering the composition of legumes and fruits that we eat, making them less nutritious.¹⁶ Further increased CO₂ is accelerating photosynthesis and making plants develop with more sugar and less calcium, protein, zinc, and other vital nutrients. Moreover, according to Harvard analysts, on the off chance that we don't diminish carbon emissions at the present time, this could spell a huge issue for our eating regimens. By the middle of the century around 175 million additional individuals have zinc insufficiency and 122 million individuals could become protein deficient because of these changes in plant physiology. This fundamental change in essential crops is a direct attack on our nutrition, which plays an elemental role in prolonging human life expectancy.¹⁷

The authors singled out income as a factor by classifying countries to yield constant and reliable results as far as possible. This is because income affects access to education, sanitation, healthcare, and diet, all of which play huge parts in extending or reducing human life expectancy. However, it is imperative to note that genetics, according to many scientists, plays an important role (about a third of variation in life expectancy is attributed to the genes: Apolipoprotein E (APOE), Forkhead box O3 (FOXO3) and Cholesterylester transfer protein (CETP))¹⁸ in determining the expectancy of an individual and it acts as a limitation to the authors' study. While the authors did divide the world into different regions

and study them separately, which allowed them to factor race and thus genetics to a degree, it still caused complications when developing valid results. Another source of bias could be the classification system of warm and cold climates allocated to each country - countries such as the United States of America and China see many different climates across their nation and while the authors classified it based on majority, it doesn't exclude the fact that discrepancies occurred. Japan is ranked as cold while Sardinia is as warm, but both are home to some of the oldest people around the world- this is due to their lifestyle factors which the authors accounted for when classifying data according to income groups, however, anomalies and variation do occur widely, such as in the case of Sardinia and Okinawa. The authors implore fellow scientists and researchers to conduct more trials to better determine the role of genetics irrespective of location and climate in affecting human life expectancy.

While conducting a thorough literature review of all the available studies on the topic of whether climate affects life expectancy, the authors came across multiple studies that further supported the results with concrete statistical and genetic evidence. A study conducted by economists from the Stanford University and the Massachusetts Institute of Technology in June 2019 collected extensive data over fifteen years of people who migrated after the age of 65 and those individuals who stayed in the same location their whole lives. This allowed them to factor out health, lifestyle, and genetics to isolate the effect of location on lifespan. The study found that life expectancy was lowest in the South of the United States (warmest parts of the country), and highest through the Upper Midwest (a much colder region).¹⁹ Additionally, according to researchers from the University of Michigan, worms that were introduced to colder temperatures triggered a genetic response that led to longer lifespans. The researchers believed that the probability of translating the same findings to humans is high since human beings have similar genetics.²⁰ Following the same line of research, scientists at Scripps Research Institute in California reduced the internal body temperatures of warm-blooded animals like mice and found that this extended their lifespans by a substantial 20.0 percent.²¹ This is corroborating evidence to the study and falls in with the quantitative measured results. Finally, when addressing climate change, researchers at the National Institute of Environmental Health Sciences found that an increase of 1 degree Celsius in temperature, increased the death rate for elderly between 2.8 and 4.0 percent.²² This is consistent with the findings, when the authors say that it is crucial to act now and reduce global temperatures.

The authors recognize individuals cannot move to colder geographic locations to theoretically increase the time they spend on this Earth. This is why the authors thought it vital to mention cold showers and cryotherapy. Most experiments that favor the effects of colder temperatures to increase life expectancy credit the explanation to either the free radical theory, or the fact that colder temperatures trigger genetic changes as observed in the study conducted at the University of Michigan. In such a case, taking cold showers or partaking

in cryotherapy regularly could trigger changes in the genetic pathways over the long term that could allow an individual in a warmer climate to enjoy the same benefits their counterparts living in geographically colder climates do.

■ Conclusion

It is clear from all data collected and quantified that individuals living in colder climates house longer-living individuals than their warmer climate counterparts. On average, when calculating the means of all life expectancies across all climate classifications and income groups, an individual may live an additional of 2.2163 years in colder regions while discrediting the role and involvement of genetics. The effects of climate change and warming of the Earth will therefore result in lower life expectancy. Further research and examination on the genetic impact of humans in different geographic locations would contribute meaningfully to this experiment. In addition, investigation of cryonics to extend the longevity of mankind, as well as preserve it can give this study a stronger grounding. Data collected and examined from research points towards the argument that human life expectancy can be extended by living in colder climates. Colder climates overwhelmingly display higher life expectancy in all four socio-economic country classifications.

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Riya Kewalani was born in 2002 in Moscow and is a graduate of Dubai Gem Private School. Kewalani is highly passionate about human biology, the climate crisis and medical ethics. She hopes to pursue medicine with biological research, and provide a valuable and positive contribution to society. Kewalani also is the co-founder of Dubai Gem Podcasts, designed to inform, inspire and ignite conversations among the youth.

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Study of Codon Usage Bias Between Genes of SARS-CoV-2 and Human Tissue-specific Genes

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ABSTRACT: Since December 2019, the SARS-CoV-2 pandemic has posed serious public health threats. Multiple organs in humans are attacked by the virus, but ways and extent to which SARS-CoV-2 affects them vary due to differing t-RNA pools. Codon usage bias of human tissue-specific genes indirectly reflects the cells' t-RNA pool for that specific organ. Once the viral codon usage bias highly matches that of genes expressed in the organ, the virus more effectively utilizes t-RNAs and causes increased damage by reducing translation of human genes. Therefore, finding such a correspondence helps in deducing organs' susceptibility to infection. Using databases and software, we compared codon usage bias of genes in the testes, uterus, brain, liver, ovaries and vulva, and the two longest genes of SARS-CoV-2: ORF1ab and Spike. We found that the two viral genes share 95% identical codon usage bias. We also found that the liver, ovaries, and testes have the most genes sharing similar codon usage bias to the two viral genes and thus a high possibility for infection. Based on Person's correlation coefficient, we identified the top three genes in the liver, ovaries, and testes. The consequences due to the reduced expression of these genes agree with clinical reports.

KEYWORDS: Microbiology, virology, SARS-CoV-2, tissue-specific genes, codon usage bias, organ's viral susceptibility.

■ Introduction

SARS-CoV-2 has been declared a public health emergency of international concern by the World Health Organization.¹ It is an enveloped, positive-sense RNA virus. The protein spikes of SARS-CoV-2 virus interact with Angiotensin-converting enzyme 2 (ACE2) on the cell membrane to infect cells.² Not only is it expressed in normal lungs, ACE2 is extensively expressed in other areas including smooth muscle cells and the endothelium of vessels in gastrointestinal tracts, brain, liver, gallbladder, kidney, heart, and testes.^{3,4} Thus, it's no surprise that multiple organs in human body are infected by the virus.

Some organs appear to be more susceptible to viral infection than the others. One possible explanation is the codon usage bias of the viral genes and human tissue-specific genes. The genetic code is degenerate. Codons that encode the same amino acid are synonymous codons. However, some codons are used preferentially over the other synonymous codons in many species. This is called codon usage bias. Codon usage bias is measured by relative synonymous codons usage (RSCU). RSCU values below 1 indicate negative codon usage biases and RSCU greater than 1 indicate positive codon usage biases.⁵

The reason for codon bias is still under study. The dominant theory of codon bias for organisms posits that preferred codons correlate to the relative abundances of iso-accepting tRNAs, thereby increasing translational efficiency.⁶ The codon bias may be important for understanding tissue differentiation. Genes selectively expressed in one human tissue can be discriminated from genes expressed in another tissue on the basis of their synonymous codon usage.⁷

Viruses need to hijack the translational machinery of the host cell for a productive infection to occur. They implement tRNA in host cells to produce viral proteins. Viruses tend

to share the same codon usage biases as at least one highly expressed protein in the disease-targeted area, emphasizing that viral and host codon usage biases are highly correlated.⁸ By studying the codon bias correlation between viral genes and tissue-specific genes, we may be able to determine which tissue a virus is likely to infect as well as which host mRNA would have limited expression.

In this research, we studied Pearson's correlation coefficient of codon bias between coding sequences of one viral gene, ORF1ab, and 169 tissue-specific genes in six organs: liver, brain, testes, ovaries, uterus, and vulva. ORF1ab was chosen because it is the first gene expressed in the host cell. The correspondence between viral and human genes is specifically defined in early viral proteins upon infection as cells undergoing the reprogramming of tRNA pools that favor the translation of late counterparts.⁹ Secondly, ORF1ab is the longest gene, thus its expression can impose great negative effects on the host cell's gene expression.¹⁰ ORF1ab is translated into pp1a and pp1b proteins. These proteins are cleaved to form 16 non-structural proteins essential for later viral reproduction, viral assembly, immune response modulation, etc.¹¹ Our study found that the ORF1ab codon usage bias is 95% identical to that of the Spike gene, the second largest viral gene. Therefore, only ORF1ab was used to represent the viral results. The liver, brain, testes, ovaries, and uterus tissue-specific genes were chosen because their codon biases show distinctive patterns.⁷

■ Results and Discussion

Organs' susceptibility to infection:

The codon usage bias correlation studies in the six chosen organs are shown in Figures 1-6.

Table 1: The number and percentage of genes with significant positive correlations in six organs, the highest correlation coefficient, and ACE2 receptor distribution.

Organ	liver	testis	ovary	vulva	brain	uterus
Total number of tissue-specific genes studied	22	21	36	42	35	13
Number of genes with significant positive correlation	14	12	15	7	0	0
Percentage of genes with significant positive correlation (%)	63.64	57.14	41.67	16.67	0	0
Highest correlation coefficient	0.77	0.77	0.81	0.70	0	0
ACE2 receptor report so far	Yes ¹²	Yes ¹²	Yes ¹³	No	Yes ¹⁴	Yes ¹³

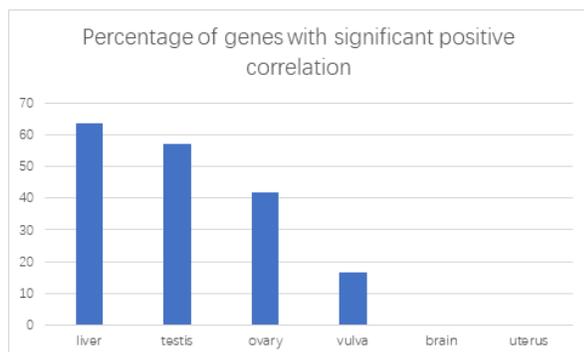


Figure 7: The percentage of genes with significant positive correlation in six organs.

Top 3 tissue-specific genes with highest correlation coefficient with viral gene ORF1ab:

The high positive correlation between the viral and human genes indicates a moderate to strong competition for the same set of tRNA and may reduce the human gene expression.

Liver:

The liver is a multi-functional organ with tasks such as bile production, nutrient storage, lipid and plasma protein synthesis, detoxification, etc.. Based on competition between viral and human genes, we speculate that the lipid metabolism, clotting factor production, and bile excretion may be affected. Additionally, there are case reports supporting the speculation on clotting factor and cholestasis.

Table 2: GI and r-values of different genes in the liver.

GI	1519394	179417	885978
r value	0.77	0.73	0.71

GI 1519394:

This GI specifies a leptin receptor. The liver is a source of plasma soluble leptin receptors and thus plays a role in modulating total circulating leptin levels and, possibly, its biological activity. One study indicates that plasma soluble leptin receptor levels were significantly associated with a lower risk of type 2 diabetes.¹⁵ Therefore reduced expression of leptin receptors in liver may increase the risk of type 2 diabetes. The liver also plays an integral role in regulating lipid metabolism.

Lack of hepatic leptin signaling may result in increased lipid accumulation in the liver and larger, more triglyceride-rich VLDL particles which may contribute to cardiovascular diseases.¹⁶

GI 179417:

Coagulation factor XIII is activated in the blood coagulation cascade. Plasma factor XIII is a heterotetramer composed of two A and two B subunits. B subunits are produced in the liver. We hypothesize that a reduced expression of coagulation factor XIIIb gene in the liver increases risk of bleeding in COVID-19 patients. In terms of reduced expression, factor XIIIb was significantly decreased in COVID-19 patient sera.¹⁷ Bleeding symptoms are not frequent in COVID-19, but there was severe bleeding in one COVID-19 patient with congenital bleeding disorders.¹⁸

GI 885978:

The SLCO1A2 gene encodes solute carrier organic anion transporter family member 1A2. This protein mediates cellular uptake of organic ions in the liver. We hypothesize that there would be a reduced expression of SLCO1A2 gene in the liver and risk of abnormal delivery of ions in the liver in COVID-19 patients. SLCO1A2 is exclusively expressed on the apical (biliary) membrane of cholangiocytes and may be involved in the reabsorption of substrates that have been excreted into the bile.¹⁹ Some liver diseases, like cholestasis, are associated with low SLCO1A2 gene expression.²⁰ Given the expression of the ACE2 receptor in cholangiocytes, the SARS-CoV-2 may enter these cells, reduce the SLCO1A2 gene expression and thus aggravate cholestasis in patients.²¹ There are reports about association between COVID-19 and cholestasis.²²

Testes:

The testes are the site of male gametes production and hormone secretion such as testosterone. Here we identify top three genes whose expression may be significantly reduced. All of the three genes are involved in spermatogenesis. The 1st two genes may interact and are implicated in microtubules and microfilaments formation while the third gene may affect energy supply. There are clinical reports showing that the sperm count, and motility is significantly reduced among the affected males.²³

Table 3: GI and r-values of different genes in the testes.

GI	1655415	755485	516123
R value	0.77	0.73	0.72

GI 1655415:

This gene encodes a molecular chaperone that is a member of the chaperonin containing TCP1 complex (CCT). The complex folds several proteins, including actin and tubulin.²⁴ With reduced gene expression the CCT complex may malfunction and the ability for cells in testes to fold actin and tubulin will decrease. Since testicular cells are supported by actin microtubule and intermediate filament-based cytoskeletons to regulate spermatogenesis during the epithelial cycle,²⁵ a decrease in actin and tubulin may lead to lower sperm production and infertility among male patients. There are reports showing seminiferous tubular injury among COVID-19 patients.²⁶ Therefore, we highly suggest that male

COVID-19 survivors should be examined for testicular and reproductive function after recovery.

GI 775485:

The IFT88 gene encodes a member of the tetratrico peptide repeat (TPR) family involved in cilium biogenesis. Testicular cilia play a role in cell signaling and tubular morphogenesis.²⁷ A low expression level will likely result in weakened cell signaling and impair tubule formation in the testes. Also, IFT88 is essential for the formation of mammalian sperm flagella and mutation results in fewer sperm and shorter flagella.²⁸ It's reported that protein product of IFT88 plays a very important role in chaperone function while CCT6B encodes a molecular chaperone. Therefore, a reduced expression of IFT88 genes could affect the protein function of CCT6B.

GI 516123:

Glycerol kinase 2 (GK2) is a key mitochondrial enzyme for regulating glycerol uptake and metabolism. GK2 is essential for the proper arrangement of crescent-shaped mitochondria to form the mitochondrial sheath during spermatogenesis in mice.²⁹ A reduced expression of this gene can lead to abnormal localization of mitochondria, causing abnormal mitochondrial sheath formation and leading to exposure of the outer dense fibers and infertility.²⁹ Therefore, in COVID-19 patients, the overall spermatogenesis could be affected, and the resulting sperm may show low motility.

Ovary:

The ovaries produce female gametes and secrete hormones such as estrogen and progesterone. Here we identify the top three genes whose expression may be significantly reduced. All three genes are expressed in follicle cells or ubiquitously expressed in the ovaries. They are all involved in follicle and oocyte maturation. While the infection effect on female fertility is controversial,¹³ but our results support a negative impact on female fertility.

Table 4: GI and r-values of different genes in the ovaries.

GI	5304925	2343116	1519394
R value	0.81	0.81	0.77

GI 5304925:

Zinc finger transcription factors are sequence-specific DNA binding proteins that regulate transcription and affect gene expression. One type of zinc finger transcription factor in follicle cells may regulate follicle maturation and competency for ovulation in *Drosophila*.³⁰ Thus, a reduction of this gene might disturb the normal follicle formation and affect later fertilization.

GI 2343116:

Karyopherin subunit alpha 5 (KPNA5) protein belongs to the importin alpha protein family and is thought to be involved in nuclear localization signal dependent protein import into the nucleus. Transcripts for KPNA5 are highly abundant in the early pre-vitellogenic stage ovaries,³¹ suggesting that KPNA5, together with other members from importin alpha protein family, may play a significant role in vitellogenesis. Since vitellogenesis occurs during the formation of primary oocytes, a decrease of its expression might lead to underdeveloped

primary oocytes and, consequently, unhealthy gametes, which can affect fertility.

GI 1519394:

Leptin is an important satiety hormone and reproductive regulator. Both leptin and its receptor are found throughout the ovaries. There is evidence indicating possible involvement in oocyte maturation, angiogenesis, follicle rupture, and/or subsequent corpus luteum formation in rats.³² Leptin may also have a direct effect on the human ovary. Leptin receptor plays a critical role in the process of ovulation by partially regulating the expression of genes involved in the preovulatory maturation of follicles in human.³³ Therefore, the presumed reduced leptin in COVID-19 female patients would affect the normal oogenesis and perturb the ovulation.

Significance:

In this study, a high correlation of codon usage bias was found between SARS-CoV-2 ORF1ab gene and tissue-specific genes of the liver, testes and ovaries. This suggests that these three organs are the target of the virus and their function should be checked during examination and reexamination. Our study also predicts the consequence of reduced expression of these human genes whose codon usage bias highly positively correlate with viral ORF1ab gene. Since SARS-CoV-2 is likely to enter those organs and compete with their genes for the tRNAs available for translation, reduced expression of those genes is a plausible prediction. Since ORF1ab is highly similar to the SARS-CoV-2 Spike gene, both genes are likely to have the same effect to the organs at different times during viral development. Thus, the two viral genes are likely to bring the same damage to the organs, making our prediction more significant. It is worth noting that this project only discussed the possible consequences during, not after, viral infection. When the virus is killed by the immune system, there will not be invaders occupying tRNAs originally used by tissue-specific genes; those genes could be expressed normally, and the symptoms could dissipate gradually.

Our investigation provides insights to potential harm of SARS-CoV-2 by studying the organ damage and the affected genes responsible for this. This project also can inform health officials about specific organs that need to be examined to ensure the physical health before and after treatment.

Limitations:

This research was conducted based on online databases, published papers, and computer operations. It lacks clinical and experimental proof for the views proposed. Also, codon usage bias is sometimes oversimplified without considerations of exceptions. Therefore, future work is needed to demonstrate the validity of our research. What's more, the ongoing mutation of the virus can make the codon usage bias more or less prominent in the future.

Conclusion

The liver, testes, and ovaries are three organs that have high correspondence to SARS-CoV-2 in terms of their codon usage bias. They are also the three organs that are most susceptible to SARS-CoV-2 infection. Abnormalities associated with reduced gene expression in the liver includes disturbed reab

reduced gene expression in the liver includes disturbed reabsorption, bleeding and disturbed leptin signaling. For testes, symptoms may include lower sperm production, shorter sperm flagella, and lower sperm motility. Male survivors should be examined for testicular and reproductive function after recovery. Possible symptoms for ovary-infected COVID-19 patients include disturbed follicle formation, disturbed normal oogenesis and ovulation, underdeveloped primary oocytes, etc. Our predictions for the liver and testes agree with clinical reports, but the viral effect on female fertility is lacking in clinical reports.²⁰

■ Methods

Sequence:

The gene IDs for genes expressed in the liver, testes, brain, and uterus is from Tissue-specific codon usage and the expression of human genes.⁴ Complete coding sequences of these genes were obtained from GenBank database (available at: <http://www.ncbi.nlm.nih.gov>). The full viral genome of SARS-CoV-2 was also obtained from the same source. (GenBank: MN908947.3)

Data analysis:

The RSCU value of the genes expressed in the chosen organs and viral genome were calculated through CodonW using the formula:²

$$RSCU_{ij} = \frac{X_{ij}}{\frac{i}{ni} \sum_{j=1}^{ni} x_{ji}}$$

X_{ij} denotes an amino acid's observed number of codons used. ni stands for the amino acid's overall sum of synonymous codons.

CodonW was written by John Peden in the lab of Paul Sharp, Department of Genetics, University of Nottingham.

Codons with an RSCU value greater than 1 are preferred in this gene and vice versa. We deleted three stop codons since they do not code for amino acids. Codons for Trp and Met were also discarded because they each have one corresponding codon and thus have no codon usage bias.

The correlation coefficients between the RSCU value of the genes expressed in different organs and ORF1ab were calculated by R software, using Person's correlation coefficient. A graph of the correlation coefficients (r) was obtained. Six graphs were obtained, representing the relationship between the codon usage bias of ORF1ab and the 6 sets of tissue-specific genes from six organs: brain, ovaries, testes, uterus, liver, and vulva.

Prediction:

We narrowed down our research to the top three tissue-specific genes with positive correlation coefficients. Then we made predictions on the consequences brought by their reduced expression.

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standing of computer software application for statistical and analytical problems in biology.

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Synthesis of PVA Encapsulated Silver Nanoparticles as a Drug Delivery System for Doxorubicin and Curcumin

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ABSTRACT: Silver nanoparticles, of great significance in drug delivery, are unstable due to agglomeration. To address this problem, we examined the effectiveness of polyvinyl alcohol (PVA) as a cheap, environmentally-friendly biomaterial in stabilizing and increasing the efficiency of drug-loaded silver nanoparticles. In this study, we have successfully synthesized *in situ* PVA-coated nanoparticles with a particle size of 70.1 nm measured by DLS and 46.7nm measured using SEM by chemical reduction method using silver nitrate (AgNO₃), sodium borohydride (NaBH₄), and PVA. Drug loading efficiency of AgNPs-PVA reached 91.95% for Doxorubicin and 99.24% for Curcumin, which is higher than the drug loading efficiency of uncoated silver nanoparticles. Additionally, we have determined the antibacterial activity of AgNPs-PVA and AgNPs-PVA-Cur on strains of gram (+) *Bacillus cereus* and gram (-) *E. coli* and a concentration of 5 ppm and above yield zones of inhibition greater than 10 mm in diameter. Given time and additional equipment, we hope to conduct further studies that examine the anticancer properties of AgNPs-PVA-Dox on cancer cells and the burn treatment applications of AgNPs-PVA-Cur on mice.

KEYWORDS: Nanomedicine; Drug Loading; PVA-coated Silver Nanoparticles; Breast Cancer Therapeutics; Burns Treatment.

■ Introduction

Over the past decades, silver nanoparticles have attracted great attention from scientists around the world due to their unique properties and wide applicability. Specifically, silver nanoparticles have been studied and used as catalysts¹ and biological probes.² In particular, thanks to their effective antimicrobial activity,^{3,4} silver nanoparticles are believed to be a potential solution to the problem of biological infection.

Throughout the world, the production of silver nanoparticles has been carried out by different methods such as chemical reduction methods, physical reduction methods, biological reduction methods, etc. The FM Gutierrez team used chemical agents to reduce silver ions to silver; the resulting silver nanoparticles were smaller than 100 nm.⁵ H. Jiang *et al.* used high-energy electromagnetic waves such as gamma rays, ultraviolet rays, and lasers to synthesize silver nanoparticles.⁶ Some studies used chemical reducing agents such as sodium borohydride,⁷ ascorbic acid⁸ to reduce silver ions to silver nanoparticles. In this study, we employed a chemical reduction method using sodium borohydride as a reducing agent because the method is the least time-consuming and requires minimal laboratory equipment.

Doxorubicin (Dox) is an active ingredient that is commonly used in breast cancer treatment today. However, Dox causes many serious side effects. Treatment with doxorubicin often leads to undesirable reactions that need to be closely monitored. Undesirable effects often depend on the route of administration, dosage, administration's speed and frequency. Nausea, vomiting and hair loss are common in many patients. Impaired bone marrow function is a very common adverse reaction to dose limits. One more side effect worth noting is

the accumulation of cardiotoxins in the patient's body.⁹ Silver nanoparticles in drug delivery, especially for Doxorubicin as a breast cancer treatment, are of great significance in the biochemistry field for their ability to bypass the cell membrane via endocytosis and to localize into the lysosomes. Here, the NPs release the drug directly, damaging cell's DNA and lead to cell death.¹⁰ Thus, AgNPs lead the drug to its destination, reducing dosage and side effects.¹¹

Curcumin is a bright yellow chemical produced by *Curcuma longa* plants. It is the principal curcuminoid of turmeric (*Curcuma longa*), a member of the ginger family, Zingiberaceae. Research by scientists in the late twentieth century has determined that curcumin plays an important role in the biological activities of turmeric. Based on *in vitro* and animal studies, scientists have proven that curcumin has antibacterial and anti-scar wound healing properties.^{12,13}

Burns are conditions of the skin due to heat damage which are very common in real life. First degree burns result in pain and reddening of the epidermis (outer layer of the skin) while second-degree burns (partial thickness burns) affect the epidermis and the dermis (lower layer of skin, causing pain, redness, swelling, and blistering which can lead to infection. At the nanoscale, silver increases its antiseptic activity by up to 50000 times greater compared with that of silver in ionic size. Silver nanoparticles can continually release silver ions and owing to electrostatic attraction and affinity to sulfur proteins, silver ions can adhere to the cell wall and cytoplasmic membrane. The adhered ions can enhance the permeability of the cytoplasmic membrane and lead to disruption of the bacterial envelope.¹³ Therefore, the use of silver nanoparticles in combination with Curcumin with its antibacterial and anti-scar wound healing

properties is also seen as a potential approach in treating burns, which are easily infected and result in severe skin scarring.

In order for nanoparticles to disperse well in the solvent without agglomeration, a coating with a surfactant or an electrostatic method that causes the nanoparticles' surface to have the same charge and repel each other is used.¹⁴ Nanoparticles formed by these methods are usually less than 100 nm in size. In this study, we examined the effectiveness of PVA as a biocompatible, water-soluble, inexpensive and non-toxic surfactant in stabilizing and increasing the efficiency of drug-loaded silver nanoparticles.¹⁵ While Doxorubicin-loaded and Curcumin-loaded nanoparticles have been previously studied such as that of Y. Du *et al.* on Doxorubicin-loaded gold nanoparticles¹⁶ and S. Ravindra *et al.* on Curcumin-loaded silver nanoparticles hydrogels¹⁷ and PVA has previously been used in combination with silver nanoparticles as a stabilizer,¹⁸ using PVA to stabilize and increase the efficiency of silver nanoparticles for loading and delivering doxorubicin and curcumin specifically is new. The first goal of our study is, by chemical reduction methods, to develop PVA-coated silver nanoparticle (AgNPs-PVA) synthesis process with NaBH_4 as the reducing agent and determine the physicochemical properties of silver nanoparticles that formed: structure, particle size, and distribution. Next, we compared the drug loading effectiveness of AgNPs-PVA for Doxorubicin and Curcumin to uncoated silver nanoparticles. The ultimate goal is to test anticancer properties of Doxorubicin-loaded AgNPs-PVA (AgNPs-PVA-Dox) on cancer cells, and antibacterial properties of Curcumin-loaded AgNPs-PVA (AgNPs-PVA-Cur) on mice and implement them for treatments on humans.

■ Result and Discussion

Analysis of Physicochemical Properties:

In this study, we proceeded to coat silver nanoparticles with polyvinyl alcohol by *in situ* method, which means that AgNPs-PVA nanoparticle synthesis process is in parallel with AgNPs granulation process.

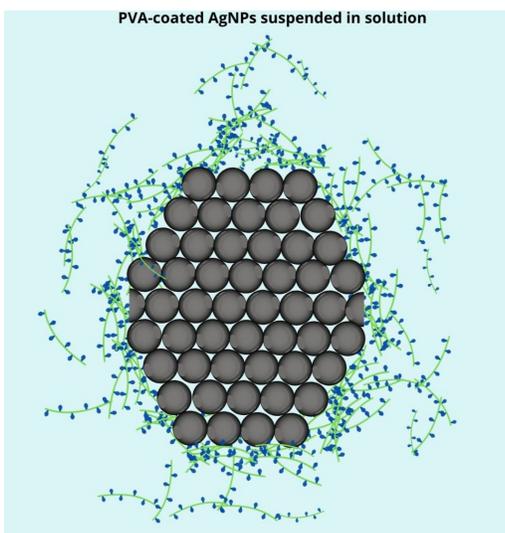


Figure 2.1: Structural properties of AgNPs-PVA.

One of the roles of PVA as a coating agent in the synthesis of silver nanoparticles is to protect them from self-aggregation in solution. Structural flexibility and conformational dynamics of a stabilizing agent backbone or a polymer chain, adsorbed onto a nanoparticle surface, play an important role in colloidal stabilization, shape-controlled growth, as well as the water-protecting effect of silver nanoparticles.¹⁹ To gain a better understanding of non-bonded interactions, occurring at the organic/inorganic interface between PVA, the AgNP core, and the surrounding water molecules, a scheme of possible interactions is given in Figure 2.1.

When conducting spectroscopy of the samples, each sample had a defined peak spectral height like the standard UV-vis spectrum of silver nanoparticles from which the silver nanoparticles could be determined. In this case, the occurrence of silver nanoparticles could be detected if the UV-vis spectrophotometer result showed the absorption peak around wavelengths of 430 nm. Simultaneously, the higher the peak is on the absorbance axis, the more of the light with this particular wavelength is absorbed, indicating higher concentration of silver nanoparticles in aqueous solution. Thus, this property helps determine if the silver nanoparticles synthesis process had happened and make preliminary prediction of the concentration of silver nanoparticles formed.²⁰

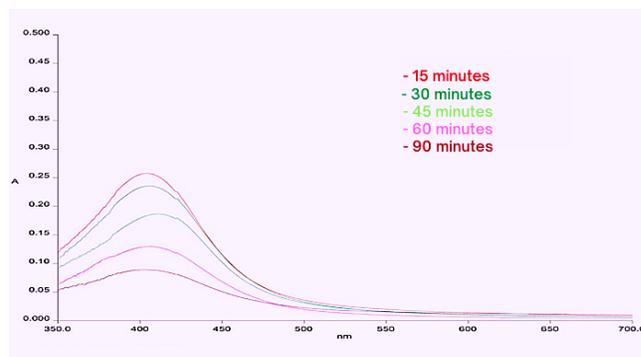


Figure 2.2: UV-Vis spectra of AgNPs-PVA solution over stirring time of 15 minutes, 30 minutes, 45 minutes, 60 minutes, and 90 minutes.

Based on this ground work, we varied the stirring time of each batch from 15 to 90 minutes in order to examine the occurrence and concentration of our silver nanoparticles suspended in water at room temperature. We measured each batch with a UV-vis spectrophotometer, whose spectrum range was set from 350 nm to 700 nm, and obtained absorption peaks at 400 to 409 nm with absorbance of 0.075 to 0.25. This result indicated that silver nanoparticles were formed although the concentration of nanoparticles in solutions was quite low. However, the most optimal stirring was determined at 15

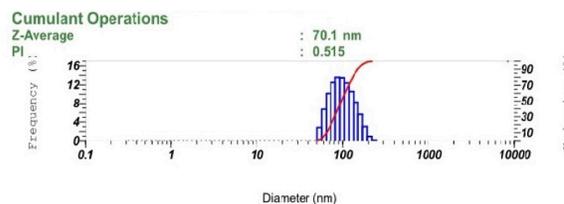


Figure 2.3: Particle size distribution of AgNPs-PVA measured by DLS.

minutes, which yielded the maximum peak wavelength at 409 nm and highest absorbance at 0.25 (Figure 2.3).

With the formation of silver nanoparticles confirmed and optimal stirring time determined, we synthesized more batches and sent them to Academy of Chemical Engineering-Vietnam Academy of Science and Technology. Our aim was to measure the particle size distribution AgNPs-PVA by dynamic light scattering (DLS), which determines a relaxation time for the decay of the autocorrelation function of the scattered light. From that, a diffusion coefficient inversely proportional to particle size can be extracted. Results of the size distribution of AgNPs-PVA nanoparticles by DLS showed that the nanoparticles formed were, in average, approximately 70.1 nm in size (Figure 2.5).

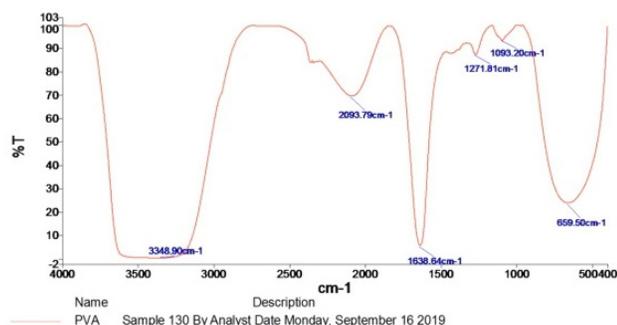


Figure 2.4: FTIR spectra of AgNPs-PVA nanoparticles.

Consequently, to confirm that the silver nanoparticles have been coated with PVA, we conducted analysis of AgNPs-PVA by FTIR spectroscopy at wavenumbers 4000 – 400 cm^{-1} . Figure 2.4 shows that FTIR spectrum of AgNPs-PVA appears to have characteristic peaks at wavenumbers 3348 cm^{-1} , 1638 cm^{-1} , 659 cm^{-1} , which corresponds to the O-H, C-C bond, and the torsional fluctuations of the OH group. This indicated the presence of PVA coating on the surface of AgNPs and bonds between PVA and AgNPs.



Figure 2.5: SEM measurement results of AgNPs-PVA.

The final method of chemical analysis we employed was scanning electron microscopy (SEM), which scans a focused electron beam over of the nanoparticles. The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition. SEM results showed that the AgNPs-PVA formed were spherical (Figure 2.5), the total number of particles was 394 and the average particle size was 46,764 nm

(Table 3.1), which is smaller than the size measured by the DLS (Figure 2.5).

The reason for the difference in the two methods lies in the mechanism of DLS, which does not measure size but, instead, diffusivities of AgNPs-PVA in an aqueous solution. If given monodisperse size distribution, i.e. all particles of the same size, then a size measurement by SEM should give a size similar to that measured by DLS. If a distribution of particle sizes exists in a solution, DLS weights the distribution differently compared to SEM, by size to the power of 6, essentially giving a z-average distribution. Larger particles are, therefore, given more weight, making the "average" size look larger than SEM's. Hence, the SEM result is a more reliable indicator of AgNPs-PVA size.

Drug Loading Efficiency of AgNPs-PVA:

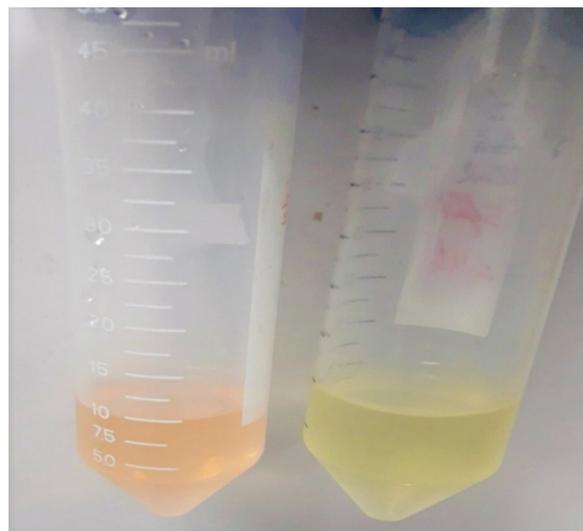


Figure 2.6: AgNPs-PVA-Dox solution (left), AgNPs-PVA-Cur (right).

We proceeded to load Doxorubicin and Curcumin onto AgNPs-PVA with concentration of 200 ppm by shaking and centrifuge. Based on Figure 2.1 in which the solid silver core is coated with dispersed PVA particles, the drug is expected to coat and be absorbed on the PVA surface of the AgNPs-PVA.

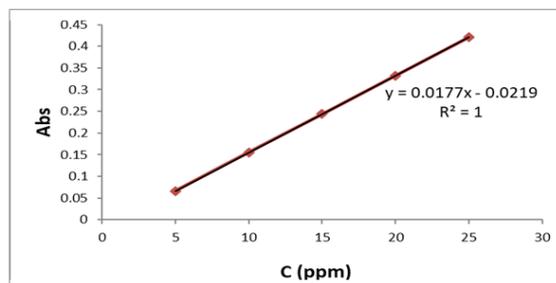


Figure 2.7: Calibration curve showing the relationship between concentration and loading efficiency of Doxorubicin.

A standard calibration curve was constructed for AgNPs-PVA-Dox and AgNPs-PVA-Cur in order to obtain the linear equation which was further used to calculate the drug-loading efficiency. We prepared stock Doxorubicin and Curcumin solutions with a concentration of 100 ppm. From these two

stock solutions, suitable dilutions were prepared at 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm. All the dilutions were analyzed by UV-Visible Spectrophotometer. The values of absorbance were then recorded and a standard curve was constructed by plotting absorbance against concentration. From this curve a regression line equation was obtained in MS Excel format.

Once the calibration curve for Doxorubicin was obtained, we analyzed our 200 ppm AgNPs-PVA-Dox, which was synthesized beforehand, by UV-Visible Spectrophotometer. The absorbance was recorded at 0.2632, which was then plotted into the equation of the calibration curve to calculate the unloaded Doxorubicin amount of 16.2090 ppm. This experimental result showed that Doxorubicin drug loading efficiency of AgNPs-PVA reached 91.9455% (Figure 2.7).

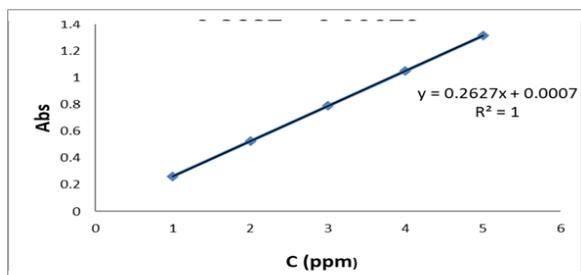
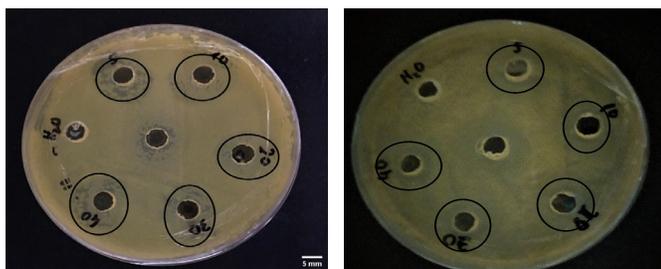


Figure 2.8: Calibration curve showing the relationship between concentration and loading efficiency of Curcumin.

Once the calibration curve for Curcumin was obtained, we analyzed our 200 ppm AgNPs-PVA-Cur, which was synthesized beforehand, by UV-Visible Spectrophotometer. The absorbance was recorded at 1.9679, which was then plotted into the equation of the calibration curve to calculate the unloaded Curcumin amount of 7.4883 ppm. This experimental result showed that Curcumin drug loading efficiency of AgNPs-PVA reached 96.2559% (Figure 2.8).

Antibacterial Properties of AgNPs-PVA and AgNPs-PVA-Cur:

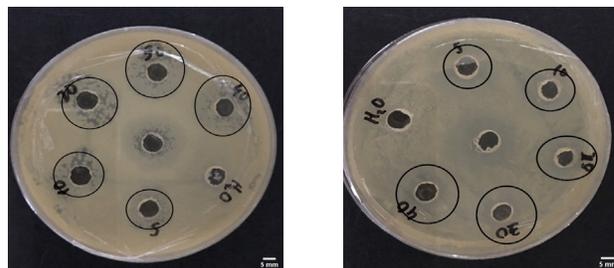


a) Bacteria *Bacillus cereus* (gram positive) b) *E. coli* bacteria (gram negative)

Figure 2.9: Petri dishes showing zones of inhibition against bacteria *Bacillus cereus* (gram positive) and *E. coli* bacteria (gram negative) for AgNPs-PVA concentrations of 5 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm.

The experimental results showed that AgNPs-PVA became antibacterial at the concentration of 5 ppm. At a concentration of 5 ppm, diameters of the zones of inhibition of AgNPs-PVA in both bacteria were greater than 10mm. The greatest diameters recorded were 20mm from the concentration of 40 ppm for *Bacillus cereus* and 20mm from the

concentration of 40 ppm for *E. coli*. This confirms the antibacterial potential of AgNPs-PVA (Figure 2.9).



a) Bacteria *Bacillus cereus* (gram positive) b) *E. coli* bacteria (gram negative)

Figure 2.10: Petri dishes showing zones of inhibition against bacteria *Bacillus cereus* (gram positive) and *E. coli* bacteria (gram negative) for AgNPs-PVA concentrations of 5 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm.

The experimental results showed that AgNPs-PVA-Cur became antibacterial at a concentration of 5 ppm. At a concentration of 5 ppm, the zone of inhibition of AgNPs-PVA-Cur is greater than 15mm. The greatest diameters recorded were 23mm from the concentration of 40 ppm for *Bacillus cereus* and 21mm from the concentration of 40 ppm for *E. coli*. This confirms the antibacterial potential of AgNPs-PVA-Cur and shows an improvement in antibacterial potential of AgNPs-PVA-Cur when compared with AgNPs-PVA (Figure 2.10).

Conclusion

In this study, we have successfully synthesized AgNPs-PVA with particle size of 70.1nm measured by DLS and 46.7nm measured by SEM. We proceeded to examine the drug loading efficiency of AgNPs-PVA for Doxorubicin and Curcumin, which reached 91.9455% and 99.2411%, respectively. We have tested the antibacterial activity of AgNPs-PVA and AgNPs-PVA-Cur on gram (+) *Bacillus cereus* and gram (-) *E. coli* for concentrations of 5 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm and found that AgNPs-PVA and AgNPs-PVA-Cur become antibacterial at the concentration of 5 ppm and above. Hence, AgNPs-PVA-Dox was found to have the potential to be tested on breast cancer cells and be implemented into breast cancer therapeutics due to their high drug loading efficiency. Furthermore, AgNPs-PVA-Cur was found to have the potential to be tested on burns spots on mice due to their antibacterial activity.

Methods

Materials:

Chemicals included silver nitrate (AgNO_3), sodium borohydride (NaBH_4), and polyvinyl alcohol (PVA) obtained from Scharlau Chemicals (Spain), Curcumin from Ho Chi Minh City University of Agriculture and Forestry, and Doxorubicin hydrochlorid (10 mg/5 ml) from Fresenius Kabi.

We used gram-positive bacteria *Bacillus cereus*, gram-negative *E. coli* from Ho Chi Minh City University of Natural Sciences to investigate the antibacterial properties of AgNPs-PVA and AgNPs-PVA-Cur.⁶

The Process of Synthesizing AgNPs-PVA from AgNO_3 , NaBH_4 :

The silver nanoparticle synthesis process was carried out as follows. A 0.001M AgNO_3 solution was dripped into 50 ml of 2% PVA solution. Then, while the mixture was being

stirred for 15 minutes in the dark, 0.001M NaBH₄ was added dropwise until the solution turned pale yellow. The reacted solution was centrifuged at 13000 rpm and washed three times with deionized distilled water. The obtained AgNPs-PVA was stored at 4°C.²¹

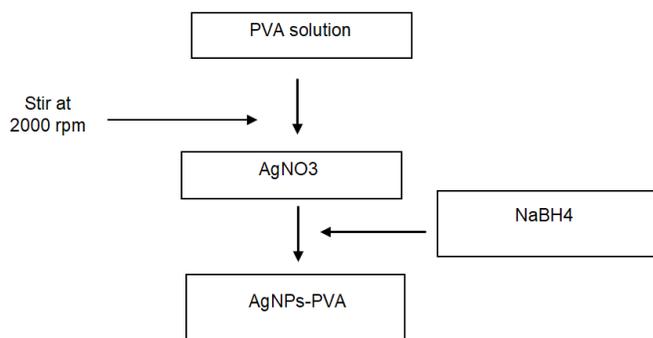


Diagram 1.1: The process of particle synthesis AgNPs- PVA.

The Process of Loading Doxorubicin and Curcumin into AgNPs-PVA:

1 ml of Dox / Cur solution (0.2 mg / mL) was added to 9 ml of AgNPs-PVA solution (2 mg / ml) at pH = 7.4. The reaction mixture was shaken with a shaker at temperature 37 ° C for 24 hours in the dark. Then, the reaction mixture was centrifuged for 15 minutes.²²

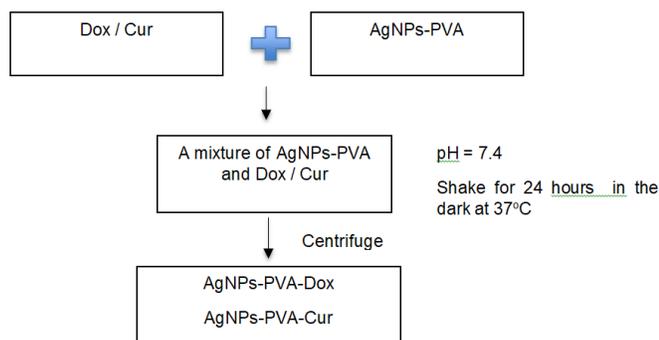


Diagram 1.2: The process of synthesizing AgNPs- PVA-Dox and AgNPs-PVA-Cur.

Chemical Analysis Methods:

Ultraviolet-visible spectroscopy (UV-Vis) is a quantitative analysis method, based on the absorption effect that occurs when matter molecules interact with electromagnetic radiation. The radiation region used in this study is the infrared region with wavelengths from 350 to 700 nm. The phenomenon of electromagnetic radiation absorption complies with the Bouguer - Lambert - Beer law. In this study, we used the model UV-Vis Biochrom S60 from Ho Chi Minh City Pedagogical University.

Dynamic light scattering (DLS) is a technique that can be used to determine the size distribution profile of small particles in suspension or polymers in solution. Fourier-transform infrared spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid, or gas. Scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning

the surface with a focused beam of electrons. In this study, we sent the silver nanoparticles to Pharmaceutical Chemistry Research Center - Vietnam Academy of Science and Technology to be analyzed by DLS, FTIR, and SEM.

Data Analysis Methods:

Particle size: we used ImageJ software to analyse the particle size of AgNPs-PVA from SEM results. The count in the section was 394 particles, with total area of 18425. Therefore, the average size of nanoparticles calculated was 46.764nm.

Drug loading efficiency:

-A standard calibration curve was constructed for AgNPs-PVA-Dox and AgNPs-PVA-Cur in order to obtain the linear equation which was further used to calculate the drug-loading efficiency. For this purpose, stock Doxorubicin and Curcumin solutions were prepared with a concentration of 100 ppm. From this stock solution, suitable dilutions were prepared at 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm. All the dilutions were analyzed by UV-Visible Spectrophotometer (UV-Vis Biochrom S60). The values of absorbance were recorded and a standard curve was constructed by plotting absorbance against

Table 1: Absorbance values of Doxorubicin solution at 1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm.

C (ppm)	Abs
5	0.0666
10	0.1551
15	0.2436
20	0.3321
25	0.4206

Table 2: Absorbance values of Curcumin solution at 1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm.

C (ppm)	Abs
1	0.26343
2	0.52613
3	0.78883
4	1.05153
5	1.31423

concentration. From this curve a regression line equation was obtained in MS Excel format.

-We proceeded to analyze our 200 ppm AgNPs-PVA-Dox/Cur, which was synthesized beforehand, by UV-Visible Spectrophotometer. The absorbance was recorded, which was then plotted into the equation of the calibration curve to calculate the unloaded amount of Doxorubicin and Curcumin. Consequently, the loading efficiencies were obtained.

-The efficiency of Doxorubicin loading is calculated by the formula below:²³

Analyzing antibacterial properties: In order to test AgNPs-PVA and AgNPs-PVA-Cur's antibacterial properties, bacteria *Bacillus cereus* (gram positive) and *E. coli* bacteria (gram negative) were cultivated in petri dishes. Next, stock AgNPs-PVA and AgNPs-PVA-Cur solutions were prepared with a concentration of 100 ppm. From this stock solution, suitable dilutions were prepared at 5 ppm, 10 ppm, 20 ppm, 30 ppm, and 40 ppm. 10g of each solution was injected into the petri dishes with corresponding amount of Doxorubicin and Cur-

Doxorubicin Loading Efficiency (%)

$$\frac{(\text{Initial amount of Doxorubicin}) - (\text{Unloaded amount of Doxorubicin})}{(\text{Initial amount of Doxorubicin})} \times 100\%$$

$$\frac{200 - 16.1090}{200} \times 100\% = 91.9455\%$$

Curcumin Loading Efficiency (%)

$$\frac{(\text{Initial amount of Curcumin}) - (\text{Unloaded amount of Curcumin})}{(\text{Initial amount of Curcumin})} \times 100\%$$

$$\frac{200 - 7.4883}{200} \times 100\% = 96.2559\%$$

cumin to be 50µg for 5 ppm, 100µg for 10 ppm, 200µg for 20 ppm, 300µg for 30 ppm, and 400µg for 4 ppm.

■ Acknowledgements

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Deepest gratitude to the Vietnam Academy of Science and Technology, Ho Chi Minh City Pedagogical University, and Ho Chi Minh University of Natural Sciences for letting us use your laboratory equipment and space.

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Spatial Distribution Patterns of Genes in Intracardiac Neurons

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ABSTRACT: Cardiovascular disease is the number one cause of deaths in the United States. Now, neural circuits are being studied to understand their functions in relation to cardiovascular diseases. Intracardiac neurons (ICN) are part of a circuit that acts as an intermediary between the vagus nerve and cardiac function. Specific ion channels have been previously identified as vital to electrical conductivity in many areas throughout the body. Assuming these ion channels exhibit similar function in the heart, the ICN can be studied for the expression of genes that encode for functions relevant to electrical activity. This study explored that if levels of gene expression for specific ion channels is assessed with respect to location in different samples of cells from a rat ICN, then a connection can be identified between the location of the specific gene expression and function. By statistically analyzing gene expression profiles from many single neurons in the rat ICN, two families of genes were identified that exhibited unique properties concerning the sinoatrial node and were functionally related to electrical activity within the body. Subsequent research will be needed to understand how an alteration in this circuit can cause differences in cardiac function.

KEYWORDS: Cellular and Molecular Biology; Neurobiology; Intracardiac Neurons; Ion channels in Intracardiac Neurons.

■ Introduction

Cardiovascular disease, be it coronary heart disease or heart attacks, is the number one cause of deaths in the United States. One person dies every 37 seconds from cardiovascular disease.¹ This is why many organizations such as the NIH have invested hundreds of millions of dollars into research on cardiovascular components such as studies related to the vagus nerve which is functionally responsible for cardiac function. Currently, neural circuits are being studied to understand their functions in relation to cardiovascular diseases. In fact, Andrew Armour, MD, PhD, has published a report showing that neuronal circuits play a role in controlling cardiac function.² Indeed, the research initiative, SPARC (Stimulating Peripheral Activity to Relieve Conditions) manipulates electrical activity in nerves for better cardiac function. Our study, which analyzes a neuronal circuit in the heart, falls under this project. Intracardiac neurons are part of a circuit that acts as an intermediary between the vagus nerve and cardiac function.² Cardiac neurons are organized into ganglia, called ganglionated plexi (GPs), where a network of 200-1000 neurons form an integration center and innervate the sinoatrial and atrioventricular nodes.¹ Functionally similar but anatomically variant, each neuron in GPs expresses genes that regulate the number of ion channels present in the ICN.³ Once DNA is transcribed in the nucleus, mRNA translation occurs in the endoplasmic reticulum and the golgi apparatus transports the ion channels to the cell's membrane where they interact with other proteins such as anchoring elements or kinases to morph into a functional channel.⁴ Ion channels are proteins that allow for the exchange of chemicals across the membrane of the cell to produce an action potential, which is an electric signal that triggers the compression of the heart.⁵ The ion channels in neurons likely contribute to the heart rate in humans. Specifically, the sinoatrial node and myocardium consist of voltage-gated sodium, calcium, and potassium

channels which contribute to neurotransmitter docking, vesicle release, and repolarization and hyperpolarization after an action potential.⁶ Neurons in the ICN are the source of the series of depolarizations which make up the action potentials powering blood vessel contractions in the sinoatrial node and in turn, contractions of the heart. 3D neuroanatomical information showing the ICN in relation to other anatomical features is necessary to understand the functional connectivity of the neurons and to elucidate their functional circuit organization.⁷ In this study, the gene expression of ion channels in intracardiac neurons in a rat heart were analyzed to support the potential relation of gene expression to location in the heart. By analyzing what genes are expressed more than others, a connection between gene expression and spatial distribution can be identified and through this, the ICN can become a target for treatments of various cardiac diseases generated by irregularities in electrical activity.

■ Methods

To begin, a model of the rat's heart was created to visualize the location of the ICN in relation to the various anatomical structures within the heart. To create a 3D model of the rat heart, a Knife Edge Scanning Microscope (KESM) was used to image sections of the heart, giving us 2D images (Figure 1). By stacking these images into TissueMapper, a custom software, the images were modified to create a 3D visualization of the rat heart. To modify the image stack, an outline was formed around all visible structures and the outline of the heart for each individual image acquired (853 in our rat heart). Next, the contours were aligned into a stack to create a 3D visualization of the rat heart (Figure 2). To do this, the aorta and the left ventricle were used to align the contours because the aorta was consistently visible throughout most of the image stack. However, when the aorta faded from view, the left ventricle appeared consistently and was therefore used. After the con-

touring and alignment was complete, a 3D structure of a rat's heart with the sampled neurons displayed in spatial relation to the anatomical structures of the heart was visible (Figure 3).

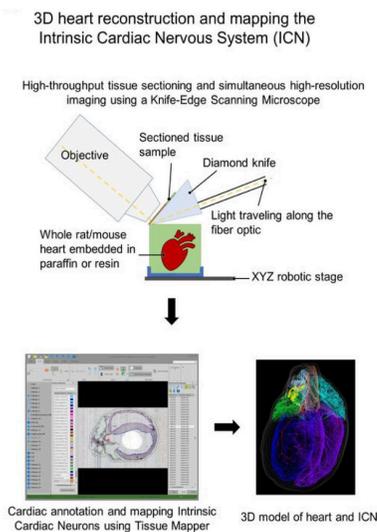


Figure 1: KESM described visually in the process of 2D imaging to later be compiled into a precise 3D model through TissueMapper.

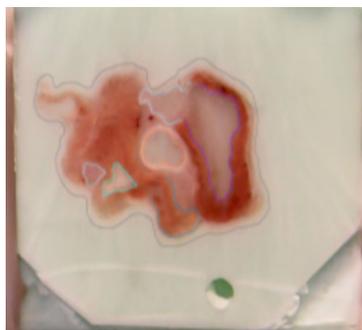


Figure 2: An image from the stack that has contours drawn outlining the interior structures and the outline of the heart.

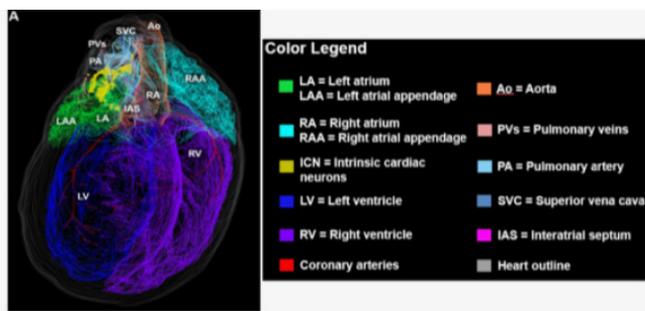


Figure 3: The 3D model of the rat heart with the ICN shown in relation to other anatomical structures. It innervates the SA and AV node, indicating a strong influence on the electrical conductivity of the heart.

To analyze the expression of genes, the raw data acquired from transcribing the RNA through RT-QPCR was normalized by finding the median among the values of gene expression and standardizing the values of 40 ion channel genes under study from various other preliminary projects compared to the median. Once normalized, the data was compiled into a '.csv' file to be readable in the R programming language. We used RStudio to run "R" and XQuartz to visualize the gene expres

sion map. Using the gene name as an input, the code produces an image of the sampled neurons colored with different shades of red and blue, showing the expression of the specific gene.

Results and Discussion

The expression data of ion channels in the ICN of a rat heart were analyzed. The experiment tested whether the gene expression of ion channels demonstrated a spatial localization specific to certain ganglia. The following images show the gene expression profile for the HCN and CACNA gene families, which play a pivotal role in electrical conductivity. Each gene is shown as a whole in a XYZ coordinate plane and an arrow points to a cluster estimated to be the sinoatrial node area.

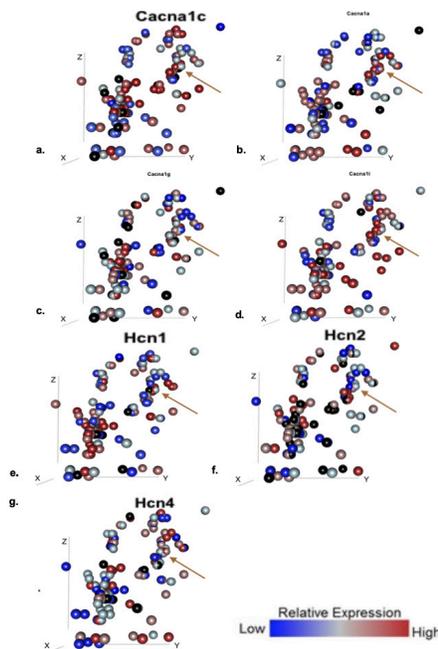


Figure 4: Gene expression maps of (a)Cacna1a (b)Cacna1c (c)Cacna1g (d)Cacna1i (e)Hcn1 (f)Hcn2 (g)Hcn4. Individual spheres represent individual sampled neurons. (h)Relative gene expression key; Extreme red signifies very high gene expression and extreme blue signifies very low gene expression. Lighter shades signify a less extreme of their respective color. Gene expression was consistently high across all selected genes in these clusters, demonstrating the select 7 genes playing a major role in regulating electrical conductivity in the heart.

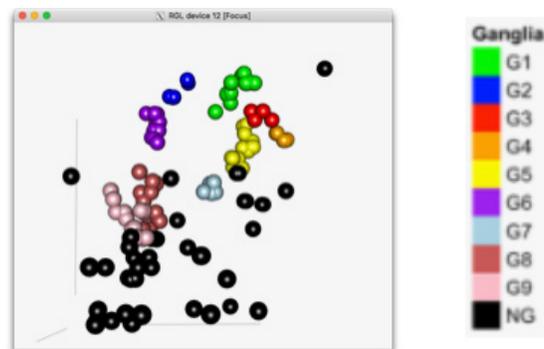


Figure 5: The separation of all 151 sampled neurons into 9 different color-coded ganglia. 3 ganglia making up the SA node cluster indicate the communicative effort of more than one ganglion to control electrical conductivity in the heart.

The neuronal circuit in the heart was analyzed specifically for certain genes encoding ion channels and receptors. While looking at the 3D images of the genes for ion channels (Figure 4), there were a handful of genes that were expressed highly around the sinoatrial node, as pointed out by arrows. The sinoatrial node is likely located in the upper right corner of the 3D images (G3, G4, G5 in Figure 5) and it is also referred to as the heart's natural pacemaker. This is because it functions as the main natural electrical stimulator of the heart, causing the heart to contract and thus keep blood oxygenated.⁸ The analyzed genes include Hcn1, Hcn2, Hcn4, Cacna1a, Cacna1c, Cacna1g, Cacna1i. These particular genes code for ion channels that have a major role in sending and transmitting electrical signals in the heart. Research shows that HCN encoded ion channels play a critical role in the generation and conduction of the electrical impulse and the physio pathological process of some cardiac diseases such as heart failure and atrial/ventricular fibrillation.⁹ The data appears to show some sort of spatial distribution observed in the genes for ion channels around the sino-atrial node, specifically HCN genes as there was a more noticeable pattern in these than CACNA genes, visually. Analyzing the images above, it could be concluded that there is a clear visual pattern in high (extreme red) gene expression in the top right corner of the images. Each gene is significantly expressed in a small region of the cluster (Figure 4).

This tells us that there is an opportunity to further investigate additional genes because there seems to be some sort of spatial pattern in this particular case. This study can further our understanding of the function of ion channels in the heart. We have extrapolated this spatial analysis across several rat and pig hearts, furthering our basis for a consistency in these specific ion channels controlling the sinoatrial node. The constant observation of similar expression in genes across multiple species and hearts eliminates several sources of errors in RNA expression when obtaining measurements. Our recordings of neurons in GPs innervating the SA node, as discovered through Z-axis slicing and microscope comparisons, differ significantly from those of other anatomical features, indicating a specialization for ion channels present in creating and containing action potentials. While precise synaptic communications cannot be measured, neurotransmitter receptors have demonstrated an imbalance in ICN, providing an opportunity for further analysis. Expanding our analysis to more genes for ion channels can also help determine additional patterns. In the future, we would like to visualize the gene expression of ion channels and other genes and compare the patterns between a healthy rat heart and a diseased rat heart. By analyzing this, we can determine the particular role that these select ion channels play in relation to the overall electrical conductance of the heart. This will allow us to determine if the neuronal circuit is disrupted in cardiac performance alterations, which can open doors for new perspectives in this work, such as manipulation.

■ Conclusion

In this study, several important markers that indicate that there is some sort of spatial distribution observed in specific genes for ion channels have been identified. This leads to the

conclusion that among the ion channels selected, there is a correlation that allows the genes for ion channels in the cardiac ICN to play a role in the electrical conductance in the heart of a rat. This analysis was also observed in other rat and pig hearts which leads to the belief that there is indeed a key role of ion channels in the cardiac neuronal circuit which regulates electrical conductivity in the heart.

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■ Author

My name is Rohan Patil, and I am a junior in high school. I have a profound interest in neuroscience because of my fascination in the power and mystery of such a system. I plan to earn an MD and practice as a pediatric neurosurgeon.

Psychological Impact of Isolation from Leader in Virtual Teams on Team Interaction

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ABSTRACT: The purpose of this study was to develop a model to show how isolation from a leader can affect employee relationships and the perception of social loafing in virtual teams. This model, assessed through qualitative interviews with virtual team employees, suggests that the effects of isolation vary from employee to employee based on the title, internal motivation, and surrounding environment. However, this model predicts that these employees will not only worsen their relationships with their teammates in the transition to a virtual setting, but they will also experience reduced relational closeness and increased uncertainty. These effects will cause employees to experience more work fatigue, put in less effort, and contribute fewer ideas (an increase in corresponding social loafing). Conversely, this research also proposes that companies are taking an increasingly active role in preventing and solving these issues to hinder the spread of these effects. The implications of these phenomena are discussed in this study.

KEYWORDS: Behavioral and Social Sciences; Sociology and Social Psychology; Employee Relationships; Virtual Teams; Social Loafing.

■ Introduction

Before the effects in the workplace are explored, it is important to define the relevant terms. The employee relationship is defined as the relationship between two or more employees, measured by collaboration and communication. Similarly, virtual teams are teams that work towards a common organizational goal primarily by utilizing technology in a virtual setting - such as Zoom, Skype, or Microsoft teams. Social loafing is often defined as the “the tendency of individuals to contribute less in a team setting” or in other words, the tendency to put in less individual efforts in a group setting due to the collective measurement of performance.¹ This phenomenon causes decreased productivity and demoralization of the hard working members that witness these members starting to complete less work. If employees begin to complete less work, organizations have less information from which to make decisions, compromising the accuracy of their decisions while hindering the advancement of the team as a whole. These varying definitions and effects will help to answer the question and narrow the scope for the interviews to ensure that the sample size is representative of the population.

The implications are numerous. If virtual teams increase team interaction and decrease social loafing, teams that have the option to work physically may switch to the virtual format, saving the company thousands of dollars in operating expenses. Moreover, researchers have previously studied the overarching topic of virtual teams, but the connection to the leader is rarely researched; further research on this connection will allow us to understand the leader's role in team interaction and social loafing.¹ Social loafing is prevalent in virtual technology because it allows employees to hide behind the screen and avoid connecting with their teammates - even if they have no prior

teamwork experience - or will not work with this virtual team after the pandemic. If employees put in less effort, team spirit and the overall productivity of the company is affected. However, results reflecting team interactions have been inconclusive as studies have stated that teams can bond more successfully depending on the tool used and the involvement of the leader. The degree of virtuality, and other team variables such as current stage, experience, and past involvement have also been discovered to play a role. However, researchers still fail to understand how distance from the leader specifically affects social loafing and team interactions.

To answer this question, we conducted a qualitative, interview-based study entailing five employees across the United States that work in virtual teams at different companies. After recording and analyzing their responses on how the isolation from the leader has affected their interactions, relationships, communication, collaboration, and contributions, we deduced the emergent patterns that materialized. Responses were expected to indicate whether the team factors increased or decreased with the team's shift to a virtual setting.

Conceptual Frameworks:

Social loafing is present in the online setting, not just in knowledge teams or technology-supported teams. Past research attributes the phenomenon to the increase in cognitive disengagement mechanisms in virtual teams: diffusion of responsibility, attribution of blame, and dehumanization.¹ Additionally, the overall structure and work environment in tech-supported industries facilitate social loafing. Employees frequently need to mentally justify the feasibility of their ideas before stating the ideas in the brainstorming phase. This creates a deficit of solid, potential ideas. In the end, this study found that dehumanization is a considerable mediator in social

loafing in virtual settings while the other two variables are mediators to a much smaller extent.¹

A major step into the research of physical isolation from the leader was a preliminary study conducted on the leader-distance theory.² The researchers found that this distance is categorized as “leader-follower physical distance, perceived social distance, and perceived task interaction frequency”. This study will explore the physical and social isolation from leaders. In technology-mediated environments virtual communication is often an asset to an organization’s leader. Other times, it is “noise” to the leader’s speech and modeling behaviors, which can decrease the effect of a leader’s inspiration on his or her employees. If a leader fails to motivate and inspire their employees, and the team refrains from partaking in “identifying responsibilities of team members, maintaining frequent contact, and promoting team-related aims,” a permanent erosion in trust and relational closeness could appear, leading to the negative exacerbation of team relationships, collaboration, and communication.²

Furthermore, researchers found that when task visibility was low, social loafing was more likely to occur due to the level of intrinsic motivation serving as the moderator.³ This implies that in organizations and virtual teams that have low task visibility, there needs to be higher levels of intrinsic motivation, like “task significance, task meaningfulness, [and] task contribution” to compensate and boost motivation.²

To elaborate, communication can be affected depending on its medium and type.³ For example, new types of technology should aid employees in fostering relationships and creating a sense of cohesion and collaboration. However, the “lean, asynchronous, communication environments” that many companies are implementing coupled with the “temporal coordination mechanism” seem to not only positively impact team relationships but also seem to prevent animosity through virtual interactions and aid conflict management.

Past research on social loafing demonstrates that virtual teams have functioned in a linear structure, suggesting that the factors are not interdependent. However, current research has proven a configurational perspective in which one effect may magnify the effect caused by another variable. For example, if team identity was present, it would magnify the level of effective communication and vice versa. If certain constructs such as team identity, collaboration, and communication are investigated, teams can transition into high-performing teams and increase relational closeness while decreasing the presence of social loafing.⁴

Similarly, social loafing can only be measured through self-feedback, but participants are not very aware of its presence. Past studies furthered research on the factors that contributed to the high level of social loafing.⁵ If the virtual team has not worked together in the past or will likely dissolve after the pandemic, employees will believe that the arrangement is temporary. This belief causes lower levels of motivation and corresponding increases in social loafing. However, researchers believed that this decrease could be mitigated with feedback to increase productivity. While productivity does increase with

feedback, the presence of social loafing still permeates virtual teams.⁵

A study explains social loafing from the perspective of mediators. Past research suggests that social loafing is affected by the familial responsibilities (the amount and type of duties that employees have in their families) and the team composition (the relative familiarity and likeliness of members in a team). The trend suggests that the higher the number of family responsibilities, the higher the degree of social loafing— although the cohesion and psychological obligation may regulate this higher level. Nonetheless, teams can still bond and familiarize themselves with each other virtually, but family activities may take precedent. In this case, due to the seniority and of the interviewees, corresponding with higher familial responsibilities, the results may be slightly skewed towards the presence of social loafing.⁶

Furthermore, the ability of employees to work with socially similar —common levels of familial responsibilities and approach to work— workers has a profound influence on their cohesion and connection to the rest of the team. “Carefree individuals who worked with mostly busy teammates perceived low levels of cohesion and obligation to their teammates and reported increased levels of social loafing in the team as virtuality increased,” but this trend is reversed when a busy teammate was to work with primarily “carefree” teammates. The extent of social loafing could vary based on the workers and their environment.⁶

Amidst the current pandemic, it will be interesting to see how the emergent of new conferencing and communicating technology such as Zoom, Skype, and Microsoft Teams will affect communication in collaboration.⁷

■ Methods

The research design is an interview-based study within which I interviewed virtual team employees to create a conceptual study on the topic. First, I reached out to contacts in varying sectors - banking, technology medicine to request an interview in July 2020.

The interview questions, presented in Table 1, were purposely open-ended to allow for more qualitative and open responses, and to capture other unnoticed trends (would not be possible with a quantifiable evaluation). Questions 3, 7, and 8 were intended to reveal the perception of social loafing from different angles and questions 2, 3, and 5 were intended to reveal the relationship between different employees (Table 1). Furthermore, questions 1, 4, and 6 were intended to capture other trends and effects of the transition that are connected to the isolation and disconnect in a virtual work environment.

Afterward, I called the various employees and conducted interviews while recording their responses through the voice memos feature to ensure that no information was unaccounted for. Then I transcribed the audio recording and analyzed the data to find potential patterns. The recording was necessary to avoid psychological biases and avoid any biases or misunderstandings in the study based on my hearing capabilities and listening comprehension. However, some questions had to be quantified to gauge the overall change between the

pre-COVID physical setting and the virtual setting, in which employees were isolated from their leader.

Table 1: Interview questions that were asked to the interviewees.

1. How do you think isolation from the leader impacts your interaction and relationships with your teammates?
2. What types of interactions are impacted by the isolation/extended virtuality?
3. How does working away from the team leader affect the performance of everybody?
4. How does virtuality impact the extent to which people collaborate and communicate in your team?
5. How did your interactions with your teammates change as a result of changing into a virtual setting?
6. How does working behind screens affect your contribution of new ideas?
7. How does working away from the group leader impact your teammates' effort towards the group goals? (explain to gauge knowledge in the role of an employee rather than a team leader)
8. On a scale from 1-10, please rate you and your teammates' motivation to contribute to the group's performance as affected by the increase in virtuality.

For the experiment, I was able to interview 5 employees at varying companies. Although this number is on the low end for the sample sizes, I used a random number generator to select these 5 employees off of a list of 50 employees. The original purpose was to establish the presence of this common phenomenon so, with 5 employees, this study will be able to pass the population proportion and the results can be considered statistically significant. The first employee was a software engineer at the highly coveted Microsoft. Due to the unique and flexible team structure at Microsoft, his perspective on the issue may be limited. Next, I interviewed a software engineer at Google. However, Google's organizational structure could impact team dynamics differently as there are multiple supervisors for one virtual team. Teams are created with some managers being leaders in some teams and just employees in others. Last but not least, I interviewed an executive at Mckinnison. His perspective may also be limited due to past involvement with his virtual team and the inherent global dispersion of team members before the pandemic - team members were already scattered all across the globe. The next member was an executive member at Pinnacle Capital Management. His perspective may be limited by the seniority of his position as president/CEO. The last was a cancer researcher for Baptist Hospital.

■ Results and Discussion

Through these interviews, several emerging factors and patterns revealed the true impact of the isolation on employees. First, based on the isolation from the leader, employees seemed to endure a lack of morale and a sense of accountability. The leaders seemed to play an instrumental role in connecting team members and cultivating morale and accountability. When workers converse about new ideas and their implementation, this culture of innovation and critical thinking holds them accountable to innovate and brainstorm ideas of their own. For example, one employee stated that he misses the "ad hoc conversations or the water cooler conversations"⁹ that allowed him to connect with the group and thus, boost his effort. Due to the isolation from the team leader, the soul of the team, this sense of accountability seems to play a mediating role to weaken efforts.

The first trend is that the research exposed was how isolation magnifies the disconnect between employees. Employees at different companies state that they are transitioning from "work buddies to more of just colleagues or coworkers".⁹ Thus, team interactions and relationships continuously diminish in

this isolated environment. Previously, the leader had played a connecting role by hosting team meetings that fostered employee-employee relationships, collaboration, and communication among team members. In the virtual format, companies are hosting fewer meetings and the leader plays less of this connecting role that is so vital to improving employee relationships and decreasing social loafing.

The second trend is that communication and collaboration have decreased but asynchronous communication has increased. Due to the nature of virtual teams, isolation from the leader has disconnected employees and caused resulting decreases in communication and collaboration; however, communication through asynchronous forms such as email and chats have increased, even pervading into after-hour periods: "beyond the regular hours from 8-5."⁹ Maybe because of the increased uncertainty about tasks and less interconnectedness, employees are taking longer to discuss issues and find solutions. Virtuality has slowed down processes and has negatively impacted team interactions, costing employees and companies higher overhead in the process.

Furthermore, there was an increase in work fatigue when working from home. Due to the fact that communication is increasingly difficult and occurred at odd times, workers are exposed to their work more than they would be regularly. One employee even stated that employees have to work more to achieve the same amount of work rather than if they were in person and sitting in a quiet environment as the "overhead is a lot to achieve the same amount of work".⁹ In other words, this extended isolation has increased work fatigue— another moderator in the study— and taken a toll on them both physically and mentally, indirectly negatively affecting their relationships with other teammates and motivation. Thus, this effect causes higher levels of effects of isolation and social loafing.

The fourth trend was that in the virtual format, team members were less motivated and were only motivated when held accountable by performance evaluation standards of their jobs. This trend could be explained by the external environment and distractions, lack of accountability and morale, or even the lack of collaboration and communication in the virtual setting. However, interestingly the dip in motivation was very minor, only about "20-25%" according to past employees.⁹ This may be due to their jobs and teams' prior relationships, but this facet needs to be explored more in-depth as both teammates showed slight decreases in their motivation: one from a "9 or 10" to an "8 or 7"⁹ and from the "8-10" range to the "7-9"¹⁰ range. Performance evaluation standards were also moderating factors as they ensured that teammates had to collaborate and communicate, increasing team relationships, and decreasing the likeliness of social loafing.

Similarly, the time after the transition to a virtual setting when the impact is measured also seems to have a distinct effect on the impact. Employees have stated that it depends on the team: some have said that "longer this goes on the more people are shying away from innovation or ideating or executing on new idea"⁹ while some have found it "quite destructive in the beginning of the whole process".¹⁰ Depending on the team stage and time measured, the results of the isolation and

the role that the leader plays can have a varying effect on the group. Thus, some teams in the performing stage have adapted and maintained levels of collaboration and communication while others in the norming stage have seen the effects of isolation magnified in their teams with worsened collaboration, communication, and relationships.

The sixth trend was that past involvement in the virtual team can affect the impact of isolation as well. This moderating variable can impact the motivation and contribution of ideas but not directly impact the relationship between teammates or the presence of social loafing. For example, teammates that had been geographically scattered before the COVID-19 pandemic experienced less of an impact from the isolation; one teammate stated that “normally the users are scattered for me and so for me, my team is scattered all over the place”.¹⁰ Furthermore, the interactions in a team can vary based on the ability of the team to work together and motivate each other: “workers need a good team to motivate each other and clearly know what the objective is”.¹¹ Without a supportive team, team members may not be held accountable or included, diminishing their motivation, and thus relationships while increasing social loafing in virtual teams. This effect could also be magnified or diminished based on the personality and individual motivation of each person on the team. Organizational structure impacts isolation as well. The flat organizational structures in Google and Microsoft compared to tall organizations in Boeing or Toyota are noteworthy because flat organizations are less likely to experience this effect of the isolation from the leader, demonstrated in the slightly more connected reviews of the employees that were interviewed. As one employee stated, “depending upon different personalities, some people really find it hard to communicate our concentrate in our conversations”⁹ which shows that extroverts or not our may be compatible and be less motivated with the online format. Others that are not technologically savvy may shy away from the use of technology to collaborate or communicate, diminishing these inter-team employee relationships and increasing social loafing. Employees that are internally driven, “whether they work at home or office they will do what it takes to get what needs to be done.”¹¹ For these employees, the isolation’s impact might not affect them to the extent that it affects other employees.

The seventh trend was that the environment seemed to have mixed consequences on team interactions and social loafing. Because many employees had high levels of household responsibilities, they spent less time on their work. Coupled with the lack of direct oversight from the leader, the employees were found to be more distracted in their work. However, some employees found this time at home to be an opportunity to work “with less distractions”¹² and focus more on work and put in longer hours. The employees that were internally driven seemed to put aside time and a quiet space for work to surpass their performance goals. Thus, the moderator of the external environment and distractions seem to result in inconclusive evidence on team interactions and social loafing due to the differing motivations and responsibilities of employees at each company. The moderating variable of hours worked also has

mixed effects- bridging employees, and closing gaps in collaboration and communication, or contrarily causing work fatigue.

However, to minimize the damage done through isolation, I have found that many companies use technology to foster communication and collaboration in virtual settings. In the 21st century, with the advent of new group communication and collaboration technologies such as Zoom, Skype, and Microsoft Teams, these technologies’ implementation would be critical more than ever. When implemented, “due to the ability of the video calls and other technological innovations, me and my teammates try to stay in touch.”¹² It seems as if the collaboration and communication have been bolstered and have not taken major hits just because of the extended virtuality. Thus, across the board technology—as a moderator— has helped teammates to interact and decreased the chances of social loafing- social loafing has been found to be present in virtual settings regardless of team, jobs, or employees.

Not only has communication and collaboration decreased in the workplace, but there have been paralleled negative effects in “sharing of the ideas”.¹² In the virtual format, employers can witness “more struggle[s] or roadblocks” in the innovation process, decreasing the mediating mechanism of the individual contribution of ideas.¹² If individuals do not contribute ideas readily, the presence of social loafing increases as employees do not want to put in more effort in the face of these roadblocks of the virtual environment. The individual contribution of ideas serves as a mediating mechanism on employee relationships and social loafing. If ideas are more willingly contributed to the virtual environment, employees are more open to contribute their own ideas, increasing connectedness with the team and decreasing social loafing. They also believe that ideas are more permanent online because they become inter-departmental in conferences, so they believe that they are obliged to ensure that their ideas are feasible and reasonable before they contribute. Finally, the brainstorming environment online is not comparable to that of the physical setting “ideas triggering off one discussion and something else is much more effective in the workplace versus in a virtual environment.”¹¹ If workers feel that their opinions will be less valued in the virtual environment and cannot even be implemented, due to the companies shutting down many projects, they will see it as futile and put in less effort in the other sectors which is indicative of social loafing.

The last trend was that employees during COVID-19 have felt more uncertain in their relationships with each other and their managers due to a loss of physical connection that was present in the workplace. This has increased the presence of social loafing. There is also uncertainty about work status, incoming tasks, and their manager’s perception of their performance. In the virtual workplace, “People are also more fearful, and they do express uncertainties,” reinforcing the divide between employees.¹² This divide causes employees to put in less work if they are not sure how their manager will perceive it, or they may have to redo or modify the project. Thus, there are increases in social loafing and decreases in the collaboration and communication between employees because of the uncertainty of the work aspects of the transition into a virtual environment.

However, many companies have acknowledged this mediating variable of individual motivation and uncertainty and have taken a proactive response: they implemented controls to ensure that workers still complete their tasks while motivating them to work towards their goals. Some companies have opted to put major projects on hold so that they do not lose money from the inactivity and increased virtual overhead while others have tried to maintain the previous levels of collaboration and communication through “regular weekly and daily meetings...if people have any issues or concerns, they can raise them, and we can solve them”.¹³ This development at least shows that even though evidence points to an increase in social loafing in the virtual format and a decrease in collaboration and communication, with the exception of asynchronous communication, companies are taking steps to increase employee motivation, happiness, and commitment throughout this challenging, virtual work environment.

Limitations:

Despite its interesting results, this study has several limitations worth mentioning. In other industries, especially blue-collar jobs, one may observe higher effects of isolation in communication and collaboration due to the more involved and authoritarian role of the leaders in those jobs. On the same note, cultural influences could influence personal motivation levels that could play a role in explaining the high levels of internal motivation, prompting lower levels of leader-follower isolation than others, and causing workers to remain motivated and connected with the rest of the team. Thus, in consideration of all of these factors, my study may slightly underestimate the true impact of isolation on collaboration and communication. First, the inclusion of younger workers and workers from other cultures may increase the effect of isolation on collaboration and communication and magnify the growing distance between employees through technology. Companies that did not heavily utilize technology before COVID-19 may lose out on the collaboration and communication that is needed for daily tasks. Lastly, since more experienced workers could have experienced a prior sense of connection and possess prior team experience, younger workers could experience the effects of diminishing collaboration and communication much quicker and stronger than the experienced workers in this study. Further research could build off of the research of the representative sample conducted in this paper and seek to corroborate these results.

Conclusion and Implications

Future research on the topic is suggested to follow a certain pattern to garner more generalizable results. First off, in the future, it would be useful if a follow-up quantitative study was performed using randomization and a large population.

In light of the research, if companies do not act on the growing divide between employees, technology, and organizations, then social loafing will continue to increase, and employee relationships will continue to worsen. The first conclusion I deduced was that collaboration and communication have decreased with a parallel divide between employees in virtual teams and organizations. Because employees may not be familiar with virtual tools or are less motivated, communication

decreases as it requires more effort to communicate the same message than in a virtual format. However, there are varying effects of this process on employees. As each employee has a different level of motivation, experience, and title, the effects of isolation such as the lack of collaboration, communication and the increase in social loafing still occur, but on different levels. Differing virtual work environments may pose more or fewer distractions and different learning styles may affect the work experience as well. However, in all scenarios, employees reduced their efforts in virtual settings. The lack of morale and motivation, coupled with the lack of a sense of accountability, contributes to the magnification of these effects. As employees possibly are less motivated and encouraged by teammates compared to the physical format through ad-hoc conversations or meetings, they are less likely to experience a sense of connection with the team and put in the necessary effort to productively function as a team.

Furthermore, employees are experiencing reduced relational and interpersonal closeness. With increased virtuality comes fewer opportunities to interact, enlarging the divide between employees. Moreover, because asynchronous communication requires both employees to talk at the same time and employees usually do not reply immediately, employees converse less often than in the physical format and have limited the conversation to work-related matters. Thus, team-wide outings and events have decreased, reducing the closeness between employees as they transition from friends into colleagues. Due to the reduced closeness and other effects of virtuality described in the paragraph above, employees are less likely to contribute ideas. This research demonstrates that employees believe that their ideas are not feasible in this time and since a declaration or introduction of ideas means that ideas are distributed officially to the entire team instead of a brainstorm, the details may have to be fully planned before. Employees may not be willing to go this extra step and think of the details, contributing to the lack of new ideas during this COVID-19 period. All of the conclusions above and in the first paragraph have contributed to the increased work fatigue in the virtual format. Employees are working more but achieving the same amount of work, increasing their work fatigue. This lack of motivation and relational closeness as well as the also increasing social loafing and reluctance to put in the extra effort has also caused them to encounter higher levels of work fatigue.

Armed with these facts, businesses have been found to take an increasingly involved role in mitigating these effects. From implementing productivity controls and performance standards to creating opportunities and resources for employees to interact, businesses are ensuring that teams can still function productively while developing relationships and contributing ideas - hence, reducing social loafing in virtual teams. Consequently, another conclusion that was found is that companies cannot fully and effectively manage their employees virtually, so they are relying on technology to do so for them - thus, automating the process and providing employees with the tools they need to succeed. Tools such as Zoom, and Slack are at use at an all-time high to preserve the level of communication,

collaboration, and introduction of ideas that occurred in the physical format.

In light of these conclusions, this research has implications for many aspects of management. First, companies are and need to continue taking advantage of the 21st-century collaboration and communication tools to ensure that collaboration and communication does not diminish in the transition to virtuality. These technological tools - Microsoft Teams, Skype, and Zoom - need to be extended to all employees so that employees communicate and collaborate. In this transition, virtual team leaders may need to play a more involved role by involving themselves in the matters of the team so that they can reduce relational distance. Companies could even host virtual events or meetings to make up for this phenomenon. Furthermore, employees do not perceive unrefined ideas as implementable and thus refrain from introducing these ideas. In the virtual format, managers may need to encourage idea-sharing and even create certain times and places to share these ideas, such as brainstorming sessions.

Furthermore, because of the increased after-hour collaboration and communication in a virtual setting, managers may need to implement procedures to restrict work after hours to decrease work fatigue from the increased asynchronous collaboration. If employee efforts can be harnessed during the work period, not only will productivity increase, but work fatigue in the virtual format will decrease. However, because research has shown that the effects of the virtual environment can vary from employee to employee and organization to organization (highly motivated individuals may persevere regardless of the environment), leaders may need to take more of an individualized approach within the team to ensure that all employees—even the ones that are not that familiar with the virtual environment—can keep up and succeed. This study adds to the growing knowledge about management as it investigates and confirms the presence of these isolationary effects in organizations amid this switch to a virtual format, prompting organizations to take mitigative actions.

However, as there was an employee that seemed to adapt well to the online environment, it can be said that the effects of virtuality may vary by person. Employees that enjoy working at home, such as introverts and employees that are more familiar with the use of technology, may harness online tools to reduce its effects and stay on pace. Thus, even amidst this shift, some employees have independently taken efforts to stay on track.

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Effect of Light and Irrigation on the Growth of *Vigna radiata* (Mung Bean) Microgreens

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ABSTRACT: There is an increasing demand for microgreens (ten-day old baby plants) globally due to their taste and nutritional values. In this study, the feasibility of growing *Vigna radiata* microgreens at home, and the effect of light (growth light [LED] vs room light [Fluorescence]) and irrigation water [10, 20, 30, 40, 50, 60 mL/day] on shoot count and hypocotyl length were determined. The experiment was conducted in plastic trays filled with soil (4 cm-depth). The results suggested that it was feasible to grow *Vigna radiata* microgreens at home under both light conditions with acceptable sensory qualities. The growth (sprouting, cotyledon development and readiness-to-harvest) was faster under the growth light condition than that of room light. However, the hypocotyl length was greater under room light than growth light. The microgreens grown under growth light were darker in color and harder in texture than room light. The hypocotyl length was significantly longer for the 60 mL/day treatment, followed by 10 mL/day treatment, and with no differences between other irrigation levels. Based on the observation of denser root systems at the bottom surface of the tray under growth light conditions, there might be insufficient soil for root development. Further research is warranted to determine the nutritional qualities of *Vigna radiata* microgreens in plots with greater soil depths.

KEYWORDS: Botany; Mung bean; Microgreens; Irrigation; LED light; Fluorescent light.

■ Introduction

Microgreens are a relatively new concept that started in the early 1980s. Microgreens are seven to ten days old small plants, harvested with two cotyledons. There has been a recent increase in the consumption of microgreen as specialty vegetables due to their taste and high content of functional components such as minerals, phenolics, vitamins, and antioxidants.¹ They are being consumed in fresh salads, soups, and other dishes. The nutritional qualities and bioactive components present in microgreens are higher than that of mature greens. Regular consumption of microgreens reduces the risk factors for obesity, cardiovascular diseases, Type 2 diabetes and certain types of cancer.²

In the official list of the American Microgreen Industries, there are 25 types of microgreens such as, *Brassica oleracea L. var. italica* (broccoli), *Eruca sativa* Mill (arugula), *Brassica rapa L. var. ruvo* (turnip), that are available commercially.² However, *V. radiata* is not available in that list. Furthermore, there is no published work available about this type of microgreen in the public domain.

Vigna radiata, popularly known as green gram or mung bean, believed to be a native crop of India, is a small green spherical shaped bean. *V. radiata* is an important legume crop in Asia, where it compliments cereal-based diets with a large proportion of digestible protein through use as a pulse. In many developing and developed countries, mung bean is used in the sprouted form.³

Since the total time required to grow microgreens is only seven to ten days, people are showing interest in growing them at home for consumption. Therefore, the optimum growth condition is required for the maximum yield of microgreens. The growing conditions have significant effects on the yield and

nutritional qualities of microgreens. Light influences the production of secondary metabolites in plants.² Several researchers have investigated the effect of different lights on the nutritional qualities of microgreens.⁴⁻⁷ Vastakaite *et al.* investigated the effect of pulsed LED during the cultivation of *Brassica rapa* (red bak choi), *Brassica juncea* (mustard) and *Brassica rapa* (tat-soi) on the phytochemical contents.⁷ It was determined that the phytochemical composition of microgreens was significantly affected by pulsed LED. Samuoliene *et al.* obtained 1.2 to 4.3 times higher concentration of chlorophylls and carotenoids in *Brassica juncea L.* (mustard), *Beta vulgaris L.* (beet), and *Petroselinum crispum mill* (parsley) microgreens under blue LED light.⁴

If the optimum growth condition is established for *V. radiata* microgreens, then it will be beneficial for the home growers. Therefore, the objective of this study was to determine the effect of light and irrigation on the number of shoots and hypocotyl length of *V. radiata* microgreens. Two types of light (growth light (LED) and room light (fluorescent)) and six levels of irrigations (10 mL, 20 mL, 30 mL, 40 mL, 50 mL and 60 mL per day) were used in this study. The experimental set up is shown in Figures 1-3.

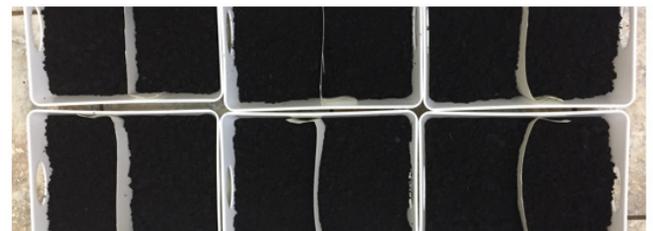


Figure 1: Preparation of tray cultivation-plots for microgreens.

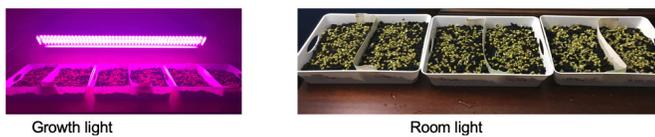


Figure 2: Experimental setup for microgreen growth.



Figure 3: Measuring length of hypocotyl.

■ Results and Discussion

The growth light showed an accelerated growth in microgreens compared to the room light. This difference was first observed on Day 2. Sprouts were noticed among the trial plots under growth lights on the second day, whereas it took four days for the room light trials. On the 6th day, many cotyledons also emerged under the growth light (Figure 4).

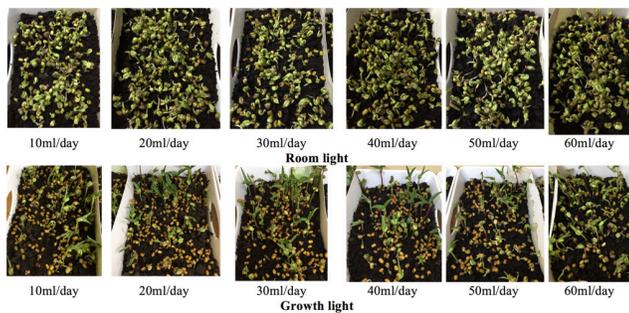


Figure 4: *V. radiata* cultivation plot on sixth day.

Harvesting:

In general, microgreens are harvested after the cotyledons are fully emerged. The seedlings should not be allowed to grow for a prolonged time as bioactive and other nutritional compounds will then be utilized for regular plant growth. In most microgreens, the growth pattern of seedlings is not uniform, therefore it may not possible to harvest them at one time. While waiting for junior microgreens, the senior microgreens might enter the regular plant phase growth (no longer microgreens). Therefore, harvesting in two stages is very common in microgreens.¹

First harvest:

Microgreens under the growth light were ready for their first harvest on the 8th day. However, the microgreens under the room light took 11 days to become ready for the harvest. The microgreens were harvested 1 mm above the soil level. The number of shoots coming from each irrigation plot was counted, and the hypocotyl length was measured immediately. Then, the microgreens were packed in zip-lock bags and stored in a refrigerator as the shelf life of microgreens is only seven to ten days.⁵ After the first harvest, the hypocotyl lengths of the remaining microgreens were short in the growth light treatments compared to the room light treatments (Figure 5). The microgreens harvested under the growth light were dark green and had a firm texture. Whereas, the microgreens were light green and had a succulent texture in the room light treatment (Figure 6).

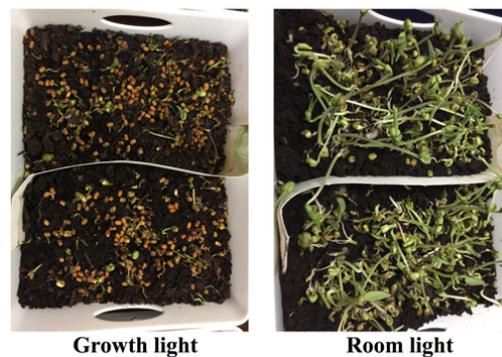


Figure 5: Cultivation plots after first harvest.



Figure 6: Harvested microgreens.

First

Second harvest:

The second harvest was carried out on the 11th day for the growth light treatment and 15th day for the room light treatment. After two harvests, the root systems were analyzed. A more extensive root system was observed in the microgreen plots grown under the growth light than the room light (Figure 7). Furthermore, the roots reached the surface of the tray and accumulated there due to lack of soil in the growth light treatments. The accelerated growth in the growth light treatment produced a more extensive root system, and thus there was no soil for further root penetration. This might have affected the hypocotyl growth. Further work under growth light with deep soil plots might yield different results than that of this study. There was no visual difference in root system between the irrigation treatments under each light.

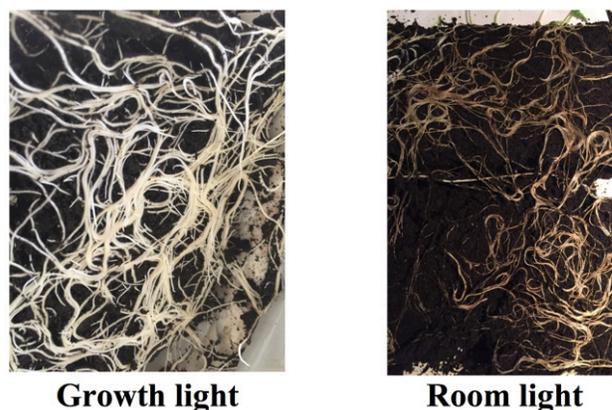


Figure 7: Root system of microgreens.

Number of shoots:

The number of shoots harvested in each treatment is shown in Table 1. Although the room light took 3 extra days for the first harvest, the number of shoots was higher (78 to 139 across irrigation levels) in comparison to the growth light (31 to 63 across irrigation levels). This trend was reversed for the second harvest. The number of shoots were 11 to 16 and 17 to 33 across irrigation levels for the room and growth light treatments, respectively during the second harvest.

Table 1: Total number of shoots.

Light		Irrigation water (mL / day)					
		10	20	30	40	50	60
LED light	First harvest	31	38	46	44	44	63
	Second harvest	24	22	24	17	33	30
	Total	55	60	70	61	77	93
Fluorescent light	First harvest	78	103	100	112	139	106
	Second harvest	13	13	11	16	11	16
	Total	91	116	111	128	150	122

Figure 8 displays the total number of shoots harvested (harvests 1 and 2) from each irrigation plot under growth light and room light treatments. The R^2 value was 0.8 for growth light and 0.6 for room light. Since the outcome of this parameter was “count”, a chi-square test (two-way analysis) was conducted. There was no significant difference on the shoot count because of the overall treatments ($p=0.14$).

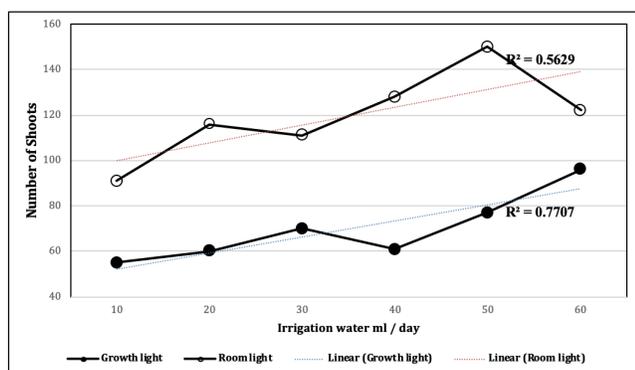


Figure 8: Total number of shoots.

Hypocotyl length:

Figure 9 shows the mean hypocotyl length (average of harvests 1 and 2) under various irrigation treatments of growth and room light. The R^2 value was low in both lights (growth light = 0.208 and room light = 0.004) compared to that of the shoot count. As two independent variables (light and irrigation) were used in this study, a two-way ANOVA was carried out for the data.

Both light and irrigation level had their own individual effect on the hypocotyl length of *Vigna radiata*. There was also a significant interaction between the selected independent variables (light type X irrigation level) on the hypocotyl length. The means of hypocotyl length of *Vigna radiata* microgreens were statistically analyzed using the least significant difference (LSD) method of comparison. The hypocotyl length of *Vigna radiata* microgreens was significantly higher for the room light than the growth light. Perhaps the room light was insufficient, and the plants were extending to obtain more light. Furthermore, the hypocotyl was significantly longer for

available on the effect of irrigation, hypocotyl length or shoot count of microgreens.⁴⁻⁶

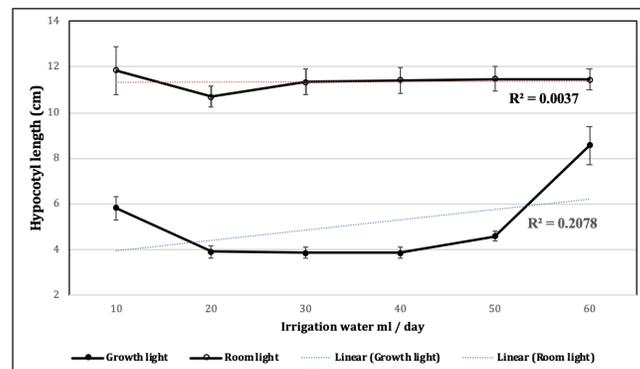


Figure 9: Hypocotyl length.

Conclusion

Vigna radiata microgreens were successfully grown at home. The growth light accelerated the growth of microgreens as observed from early sprouting, early cotyledon development, and harvest dates compared to the room light. However, the hypocotyl length of the microgreens was shorter in the growth light than that of room light treatment. As an intensive root system was observed near the tray's surface in the growth light treatment, the growth might have been affected due to lack of soil for penetration. Both tested independent variables (light type and irrigation level) had significant effects on the hypocotyl length of the microgreens. A sensory evaluation determined that microgreens grown under growth and room lights were different in texture and appearance. A darker color and firmer texture was noticed from the growth light treated microgreens, and those harvested from the room light were a lighter color and had a succulent texture.

Future work:

Further work is warranted to determine the growth of *Vigna radiata* microgreens under growth light without any soil depth limitation, including the dry weight of the plant. Studies for the effect of root development on texture, color and taste of the microgreen would be beneficial for optimizing light and irrigation levels. A detailed sensory evaluation will yield an approximation of consumers' preference for microgreens grown at home. Based on the consumer's preference and nutritional qualities, growth conditions such as light type, irrigation quantity and soil depth could be optimized. Since microgreens are relatively easy to grow at home and rich in nutrition, people should be encouraged to grow and consume *Vigna radiata* microgreens in their homes.

Methods

Lights:

LED light (Byingo (2ft, 32W) plant growth light-Full spectrum for indoor plants) was purchased through Amazon. ca. and used in this study. Experiments were conducted in two separate rooms, one with uninterrupted LED lights and the other with uninterrupted fluorescent lights. They were the only lights available in these rooms, which were kept closed all times during the experiments to avoid light interaction from other rooms.

Room temperature:

Temperature affects the growth rate of microgreens. Since temperature was not used as an independent variable in this investigation, it was kept constant in all treatments, at approximately 20°C.

Other materials:

The size of the plastic tray used in this study was 26.6 x 20.9 x 6 cm. Water absorption will vary when different quantities of soil are used. Less water will be available for *V. radiata* germination if there is a greater depth of soil, as water will evenly diffuse throughout the soil. By keeping these factors in mind, 500 g soil was used for each treatment, and the depth was approximately 4 cm. The soil contained a combination of humus, compost, and Canadian sphagnum peat moss. Fifty grams of *V. radiata* seeds were sowed for each treatment. The seeds were submerged in water for 24 hours before sowing. The hypocotyl length was measured using a measuring tape as mentioned in Figure 3.

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Mathematical Analysis: How Would AI Tackle Math Olympiad Problems?

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ABSTRACT: Artificial Intelligence (AI) has increasingly gained attention as a novel approach to problem solving. Computer scientists are currently in a race to build an AI system that will win a gold medal at the world's premier math competition, the International Mathematical Olympiad (IMO). In order to succeed, it is critical to design and evaluate a way to assess how AI undertakes Olympiad questions. This paper examines the ways in which students are evaluated for their mathematical skills and suggests that such assessment be implemented for the AI in order for it to successfully solve math Olympiad questions. The challenge lies in determining a method of applying mathematical creativity and thinking ability to AI.

KEYWORDS: Mathematics; Other; Artificial Intelligence; Problem Solving; International Mathematical Olympiad.

■ Introduction

The fields of engineering and natural sciences have contributed significantly to the development of modern technology. Among many, mathematics has had the most crucial impacts on technology because it has helped humans think logically, critically, and innovatively. Therefore, to master mathematics well, the government establishes mathematics as one of the fields of science studied at every level of education from elementary school to college in most countries.¹

As mathematics is highly valued, there has been many attempts to assess one's understanding. However, it has remained a difficult task to find valid and standardized instruments that can identify those gifted in mathematics. After long years of research, mathematicians have come to a conclusion that mathematical creativity and thinking ability are the fundamental requirements to be a talented mathematician.² And now, computer scientists are taking this a step further and trying to build an Artificial Intelligence (AI) system that can win a gold medal at the world's premier math competition, the International Mathematical Olympiad (IMO).³ To achieve this, it is critical to design and evaluate an approach that will assess how AI undertakes Olympiad questions.

■ Results and Discussion

Values Required for Olympiad Problems:

According to Kim and Cho, mathematical creativity is like a plan and it contains four main aspects: fluency, flexibility, originality, and elaboration.² In their explanations, fluency addresses how well the person can generate and create multiple responses, while flexibility addresses how well the person can generate different categories of responses that are not fixed on just one idea. Originality describes how unique the responses are, while elaboration describes how extensive a person can develop a simple design into a more complex one.²

On the other hand, mathematical thinking ability can be more like a set of techniques that help you execute a plan. Thinking abilities are composed of several sub-abilities includ-

ing intuitive insight (picking up critical cues in the problem), organization of knowledge necessary for solving the problem, visualization of the given information, abstraction of the problem into concepts, symbols, formulas, and figures, reasoning through inductive and deductive thinking, generalization of the mathematical relationships, and reflection of own problem-solving instances in the past.²

Questions in mathematical competitions such as the IMO often require both mathematical creativity and thinking abilities at the same time. Thus, the biggest task is to educate AI so that it can apply both mathematical creativities and thinking abilities appropriately.

How to Perceive and Solve Olympiad Questions

A. Overview

Solving mathematical problems is similar to hunting for the hidden treasure; there is a final goal to reach and several clues are given upfront. For easy questions, the information is too obvious to miss, or the amount of information given is more than enough. However, the more challenging questions in the IMO will give you indirect clues and these will often be ill-defined so that the clues are not easily noticed. At the same time, many different types of questions will cover a wide and high level of mathematics and demand both creativity and thinking ability.

There is always a good place to start no matter how complicated a question is and how peculiar its topic is. Usually, problem-solving strategies such as heuristics, is very effective in making progress on unfamiliar and non-standard problems without evident clues. According to Cheung, one should first understand the problem and then devise, carry out, and revise the plan to tackle unfriendly problems successfully.⁴

In most cases, searching for a pattern is a good start because it could lead to a conjecture or even a proof. Sometimes, few modifications can be made to a question in order to find the pattern. Simply, the question can be restated in terms of simpler words or mathematical symbols if it contains too many

difficult terms that confuse you. Once the question is turned into a similar but easier one, a pattern can be more easily noted.

Although heuristics are handy in understanding and analyzing problems, they often end up providing tedious and possibly inefficient solutions. In other words, the most efficient and interesting strategies are topic-oriented and interdisciplinary. Therefore, a skilled student should be able to apply different approaches for different types of problems. The below examples are some of the popular types of approaches that are categorized and analyzed by Cheung.⁴

B. Methods of Proof:

Proof problems are easily found in the IMO, but most students often find them tricky. The reason why students find the proof problems difficult is because it takes more knowledge, discipline, and effort to prove a general statement than to prove only a single case. Unlike what is expected, general methods of proof usually do not require multiple proofs to prove a proposition to be true. Instead, they involve two different proofs with connecting logic.

There are two major approaches to proof problems: induction and deduction. Proof by induction is very common in Olympiad problems. As mathematical induction is a technique for proving a statement for natural numbers only, if one can easily prove a statement; the statement should then be proven to be true for the first iteration and its $(n+1)^{\text{th}}$ iteration is then proven to be true when the statement still holds for the n^{th} case. Contrarily, proof by deduction establishes a true proposition by providing well-known mathematical principles that have already been found true. It often seems to be easier to employ proof by deduction, but it requires more caution as one needs to make sure certain principles are appropriate and applicable to specific cases.

C. Strategies on Counting:

Problems on counting are common in mathematical competitions and there are a couple of strategies to tackle such questions.

The pigeonhole principle is one of the most well-known and commonly used principles. The principle states that if n items are to be put into m containers and n is greater than m , then at least one container contains more than one item. This seemingly obvious principle is extremely useful when a question involves matching items or minimum number of trials.

The next helpful technique is the principle of inclusion and exclusion. The basic idea of the principle is that adding all the number of elements that satisfy at least one of several conditions and subtracting all the overlaps, then, any element would not be accounted more than once. The simplest case would be the following.

D. Geometry:

Unlike algebraic problems, there is almost no limit to the variety in geometric problems. Simply, there are infinitely many dots, lines, faces, and three-dimensional figures in three-dimensional space. There is always a general approach

to geometric problems, but it is often topic-oriented and unfortunately inefficient most of the time.

As mentioned above, breaking a complex polygon down to simpler pieces such as triangles and circles would be a decent start. The properties of a triangle are fairly simple but useful and so are those of a circle. Therefore, using their properties might lead to another essential equation and so on. For some cases, the properties of different types of quadrilaterals could be helpful but those of quadrilaterals are generally not as useful or not applicable to most of the cases.

E. Synthesis versus Analysis:

Cheung not only provided useful strategies to various types of Olympiad problems, but also analyzed different general approaches.⁴ In proving equations or inequalities, synthesis and analysis are the two fundamental techniques to solve such problems.

If the equation or inequality is solved synthetically, the given condition is modified multiple times using basic mathematical principles to deduce the final equation or inequality. Mostly, the basic principles are applicable unconditionally but there are often cases where some restrictions should be made in order to employ modifications based on the basic principles.

While a synthetic approach starts from given conditions, building up adequate relations, and eventually deduce a final solution, the analytic approach is exactly the opposite. In an analytic approach, the final equation or inequality is altered to obtain the initial conditions. In other words, synthesis is to figure out necessary conditions for the given condition whereas analysis looks for a sufficient condition for the final equation or inequality. Most importantly, every deduced step in both processes should be reversible.

F. Partial Attack versus Global Views:

Just like the case of synthesis and analysis above, partial attack and global view are simply different perspectives.

Partial attack is useful when a general relation should be concluded, and few pieces of information are given initially. This approach is naturally used in finding a general formula, for example, finding the general n^{th} term of a geometric sequence. The common ratio could easily be calculated if any two terms of the sequence are known. Accordingly, the value of the first term can also be found since the ratio is known. Then, the general formula for n^{th} term of the sequence, a_n , would be $a_n = a_1 r^{n-1}$ where a_1 is the first term and r is the common ratio.

The global view is technically the opposite of the partial attack. Appropriate equations and relations can be applied to calculate a solution for specific conditions. This can be as simple as using the quadratic formula to calculate the roots for a specific quadratic equation. Indeed, Olympiad problems are much more complicated than this.

How to Evaluate and Improve One's Competence for Solving Olympiad Questions

A. Overview:

Using the strategies suggested above, a student can possibly tackle many Olympiad problems successfully. However, there can be a number of steps that could go wrong in the middle of the so-called strategies. For instance, the student might not understand the problem at all, not know the purpose of

the problem, lack planning skills, not know the concepts and formulae that must be used to solve the question, and simply make mistakes. According to Hidatati's research, applying appropriate but incorrect formulae and applying random formulae were the most common errors.¹

B. Cognitive Conflict Strategy:

In order to minimize such errors, cognitive conflict strategy was applied to improve students' mathematical critical thinking ability.⁵ If a student makes a mistake, he or she is given relevant counter-information, an analogy, or relevant guided questions that challenge his or her solution. Given the information, he or she is encouraged to go over his/her own answer and hopefully they figure out what went wrong. This way of learning, cognitive conflict strategy, has helped many students think critically and realistically. Furthermore, it has made the students more positive and enthusiastic.⁵

C. Analogy in Scientific Thinking :

Clement has analyzed a problem-solving protocol and found the role of analogy in scientific thinking.⁶ Given the initial conception A, Clement emphasized that a wide range of knowledge is required to come up with an analogous conception B, and the conception B and the analogous relation must be confirmed.⁶ Once the analogy is established, it can be used to solve unfamiliar problems; they can be related to several analogous ones that are more familiar. As long as the modified problems are taken care of, the original problems can be revised in a similar manner.

D. Scientific and Problem-Solving Approach :

For the process of solving a question in general, two basic methods exist: the scientific approach and problem-solving approach. The scientific approach refers to an empirical method to construct a formula or a principle. To learn scientifically, one should observe a problem, build a hypothesis, test if the hypothesis holds, and conclude the relation. Thus, the scientific approach is useful in developing a formula in general while the problem-solving approach focuses more on individual cases. In other words, one should identify the problem, define the problem, explore the solution, act on a strategy, come up with a conclusion and review the problem.⁷ The problems that use the scientific approach are characterized by the number of trials are required until hypothesis is proven to be true. Contrarily, a number of true cases should be obtained to establish a general solution for the problem-solving approach. According to Chairuddin, students that practiced the scientific approach scored about 6 % higher mean scores than those practiced the problem-solving approach.⁷

E. Development of Metacognitive Level :

The higher the metacognitive level, the greater the creative thinking abilities. As students' metacognitive abilities develop, they are not only aware of their use but will progressively use these abilities strategically and will reflect back and evaluate these abilities.⁸

Student's metacognitive skill develops with age. With repetition, maturity, biological development, and good Olympic training, they will be able to improve on their creative thinking abilities and behavior.⁸

Students must not fixate on the concepts taught in school but must be continually exposed to multiple forms of questions to promote their creative thinking skills.⁸

Progress of AI and its Current Location

A. Overview:

While we have been flooded with a tremendous amount of decent AI applications in recent years, machines are still not good enough to understand problems, provide appropriate answers, and have serious discussions with others. To secure the next level of AI applications, it is important to understand the history, realize the current issues, know the principles and apply them properly to come up with advanced solutions. Only then, AI that can solve Olympiad problems can be invented.⁹

B. History and Application of AI:

In 1936, Alan Turing, a British mathematician, invented a Turing machine that manipulates symbols on a strip of tape based on pre-made principles. Consequently, he published an article about how to test if machines can think, if machines can learn, and how intelligent machines can be developed in a paper called *Computing Machinery and Intelligence* in 1950. Later, his work fostered the standard for modern computer technology and is seen as the beginning of the history of artificial intelligence.¹⁰

Despite Alan's early contribution to artificial intelligence, the term artificial intelligence first appeared a few years later, in a meeting known as the Dartmouth Conference in 1956. John McCarthy, a mathematician and scientist at Dartmouth College, planned a summer research project on artificial intelligence and invited his fellow researchers. In the invitation letter, he wrote "We propose that a 2 month, 10-man study of artificial intelligence be carried out during the summer of 1956 at Dartmouth College in Hanover, New Hampshire."¹¹ At this meeting, the participants discussed how to embody Turing's "thinking machine" and transform it into a system of logic and form.

Since then, early AI scholars have shown a high level of trust and confidence in the potential of artificial intelligence. Allen Newell and Herbert Alexander Simon said that computers would be the champions of chess and the computers would be able to compose music of aesthetic values. Furthermore, Herbert even stressed that machines would be able to do whatever humans can do in the next couple of decades.¹³

Although it fell short of his prophecy, remarkable progress was made over the next 20 years. Artificial intelligence research centers were established at MIT and Carnegie Mellon University and a number of possibilities were studied. In 1959, Simon and Alan created a general problem-solving algorithm that was able to solve the "Top of Hanoi" puzzle.¹³

Later, research on artificial intelligence had been split into smaller topics and started to focus on practical purposes. The 'experts system' has been introduced and it refers to a system that organizes, expresses, and stores expert knowledge possessed by humans in a specific field, so that the general public can use the knowledge. A few good examples are the following: Logic Theorist of Carnegie-Mellon University in 1956, DENDRAL, a molecular structure identification pro

gram created in the 1960s, and Mycin, a program designed to diagnose patients with infectious blood diseases and prescribe antibiotics.¹⁴

During the 2000s, based on Professor Hinton's Deep Belief Network, deep neural network (deep learning) technology began to show potential for practical use. In particular, Deep-CNN (Convolution Neural Network: specialized model for image recognition/classification) showed a 26 % recognition error rate.¹⁵

As soon as computers could perceive problems more accurately and effectively, their performance has soared abruptly. Google's AlphaGo 1.0 beat a Go world champion, Sedol Lee, in 2016 and AlphaGo 2.0, the master version of AlphaGo 1.0, beat Ke Jie, the number one ranked player at the time, at the 2017 Future of Go Summit. Even at this moment, scientists and engineers have computers collect and study large amounts of data and research what fields and applications artificial intelligence can be used to enrich human life.

■ Conclusion

In order for AI to successfully solve math Olympiad questions, one must learn how to educate AI so that it is capable of utilizing mathematical creativity and thinking abilities. This paper examines the ways in which students can be assessed for their mathematical skills and suggests that such assessment be made for AI to possibly develop AI capable of solving math Olympiad questions. In the past, the four-color theorem was finally approved by mathematicians once they developed a computer model to assist the proof. At first, this theorem, which states that only four colors are needed to color the regions of a map such that no two neighboring regions sharing a common boundary have the same color, was extremely difficult to prove, and many mathematicians held opposing views. However, the conflict was resolved once researchers developed a computer model to prove the theorem. Similar to this, the seemingly challenging math Olympiad questions may also be tackled by AI. In order to achieve this, this paper suggests that it is critical to understand the foundations behind students' learning and problem-solving skills and to devise a way to implement such skills in AI. Previously, researchers at Cornell University successfully devised open Internet collaboration on mathematical research problems.⁹

As seen in the examples of AlphaZero and AlphaGo, AI has outperformed humans as soon as they fully understood the rules of Go or Chess. Likewise, this paper has sorted some common types of mathematics Olympiad problems and provided basic approaches to them in order to help AI understand and eventually solve the problems by itself. Hopefully, this paper will lay the foundation for application of AI for solving mathematical problems, and consequently, AI systems that solve mathematics Olympiad questions themselves accurately will soon follow.

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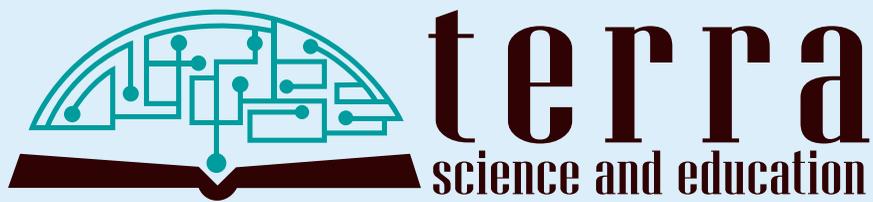
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