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Ex vivo Nitrate Exposure, DNA Damage Indicators, and Cytokine Response of Peripheral Blood Mononuclear Cells

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Abstract: Nitrogen in the form of NO is a critical biomolecule (Halliwell & Gutteridge, 2007). However, when excessive amounts of agriculturally derived, soluble nitrogen contaminate drinking water supplies and reach high concentrations in human body fluids, reactive nitrogen can create both acute and chronic health concerns. This study examined lymphocytes in-vitro, examining varying exposures of nitrite (in the form of sodium nitrite at 1, 5, 10, 15 ppm) and lymphocyte reactions to these exposures from the perspective of inflammatory cytokine expression and indicators of DNA stress and damage such as evidence of induction pathway dephosphorylation and histone indicators of DNA strand breakage. This study illustrated that IL-1, IL-6, IL-17, TNF-α, and IL-10 cytokine expression in the majority of exposure cohorts fluctuated in a statistically significant fashion, often in a directly proportional manner, especially in cohorts of 1-10 ppm nitrite exposure, that DNA damage as indicated by the percentages of cells exhibiting PH2AX, ATM, DNA [histone expression] increase in response to increases in nitrite exposure, and that these increases in cytokine expression and DNA damage were seen irrespective of demographic characteristic or health status. These findings indicate the need for further study in larger population samples and pose interesting questions concerning chronic disease processes and nitrate/nitrite exposures via drinking water.

Keywords: Nitrite, Cytokine, DNA, Chronic Disease, Inflammation

Introduction

The nitrogen molecule in its soluble and reactive forms plays an essential role in both the natural environment and a variety of human physiological processes, being both essential for life and posing risks to life. Reactive nitrogen has a liminal nature in the natural environment and physiological systems, it is necessary for life in

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certain forms (i.e. NO) and amounts and creates physiological toxicities in other forms and amounts (i.e. NO3-, NO2-).

Nitrogen in its derivative forms of nitrate, nitrite, ammonium and nitrogen oxide in natural ecosystems, provides the essential elements for productive plant growth, forming important water-soluble nitrogen oxides. New research points to a possible stalling of this naturally available and naturally "fixed" form of nitrogen in terrestrial ecosystems, due to disruptions in carbon cycling and consequent climate change (Mason et. al. 2022). Nitrate/Nitrogen is present in the aquatic environment at levels ≤ 2 ppm nitrate-nitrogen in areas without significant human agricultural activities. However, environments impacted by agriculture or animal husbandry associated uses are experiencing much higher levels of nitrate-nitrogen loading to drinking water sources, often far exceeding natural background levels of 2 ppm nitrate-nitrogen, requiring that many municipalities implement additional, costly water treatment regimens.

Since the 1970s, the main driver of the soluble nitrogen cycle in agricultural systems has been anthropogenic inputs, driven by the Haber process, so that total teragrams/year of human inputs to agricultural lands now easily exceeds 200 Tg/yr (Spiro et al., 2011). Therefore, a paradoxical situation appears to be developing wherein terrestrial ecosystems not associated with human agricultural activities are "starving" for reactive nitrogen, while human managed, agriculturally influenced watersheds are receiving excessive amounts of soluble, reactive nitrogen inputs. It is the human health impacts of this disrupted biogeochemical cycle that this paper explores.

Background

Nitrogen in the form of NO is a critical biomolecule (Halliwell & Gutteridge, 2007). However, when excessive amounts of agriculturally derived, soluble nitrogen contaminate drinking water supplies and reach high concentrations in human body fluids, reactive nitrogen can acutely impact the function of the red blood cell, leading to health problems in infants and adults, namely, chemically induced methemoglobinemia, MHG. MHG is a condition wherein the red blood cell cannot properly bind with oxygen, leading to hypoxia. Further, reactive nitrate exposures through drinking water are epidemiologically associated with cancers and other chronic disease issues, according to the findings of well over three dozen epidemiological studies since the mid-1970s, ranging in design from ecological to case control and prospective cohort (Zeman, 2019). Additionally, it has been associated in some biomolecular studies with DNA damage, namely chromatid breaks and mutagenic changes in Ames assays research dating back to the late 1990s (Osoata et. al., 2009; Tsezou et. al., 1996; vanMaaneen et. al., 1996). The interplay between inflammatory states, induction pathway disruption, and DNA damage could help explain some of the chronic disease and epidemiological findings in this area and was a focus of this research. One pathway to studying this is to examine lymphocytes in-vitro, examining varying exposures of nitrite (in the form of sodium nitrite) and lymphocyte reactions to these exposures from the perspective of inflammatory cytokine expression and indicators of DNA stress and damage such as evidence of induction pathway dephosphorylation and histone indicators of DNA strand breakage.

Cytokines are immune messenger molecules, produced by cells of the immune system, especially the various subsets of T helper lymphocytes, including the proinflammatory Th1, Th17, and the anti-inflammatory T-regulatory, Treg and Th2 T-cells. Monocytes/macrophages, T-killer cells, natural killer cells (NK), and dendritic cells also produce some cytokines. Cytokines have a wide range of functions, some of which oppose the action of other cytokines. Cytokines can both encourage and decrease inflammation, depending on the cytokine, for example, pro-inflammatory cytokines include interleukin 1 (IL-1), tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and interleukin 17 (IL-17), while other cytokines, such as interleukin 10 (IL-10), down-regulate the immune response so as to halt inflammatory processes at the appropriate time, maintaining homeostasis (Helbert, 2017).

Inflammation is a critical defense against microbial infection and critical for wound repair when present in the correct degree, location, and time frame. However, when excessive and chronic inflammatory processes are present, the foundation is laid for many chronic disease processes (Helbert, 2017). Chronic, pathogenic inflammation may occur in many tissues and organs and can lead to mild or severe disease. Many anti-inflammatory therapeutic drugs act by downregulating the levels of pro-inflammatory cytokines, dampening the inflammatory response. This too has risks as it decreases the immune response to viruses or bacteria, as evidenced by the warnings about severe or possibly fatal infections included in advertisements for drugs acting to down regulate proinflammatory cytokines. Individuals with down-regulated immunity or suppressed immunity are at risk of developing numerous infections such as tuberculosis and fungal infection by histoplasmosis. It is important, therefore, to maintain a fine control over the balance of pro-inflammatory and anti-inflammatory cytokines.

Guided by insights and questions derived from previous work, Peripheral Blood Mononuclear Cells (PBMC) from osteoarthritis sufferers and from healthy controls were used in this research. PBMCs contain T helper and killer lymphocytes, B lymphocytes, monocytes/macrophages, and NK cells, but are primarily composed of T cells. Osteoarthritis is a chronic synovial joint inflammatory condition that, unlike rheumatoid arthritis, is not classified as an autoimmune disease (Zeman et al., 2011).

Earlier work by members of our research group found that exposure to NO2, either in vitro or in ingested well water, decreases proliferation of T lymphocytes from normal donors in a dose-dependent manner, in addition to altering cytokine production in a manner that varies among individuals (Ustygova et al., 2002; Zeman et al., 2011). These mixed results indicate that other modulation conditions are present in select participants. A cohort study examining cytokine levels from people consuming differing amounts of nitrate in their well water found a linkage between IL-10 production and joint pain from osteoarthritis as well as gastrointestinal conditions (Zeman et al., 2011). That study examined the health parameters and ex vivo (in the body) immune system functions in a cohort of persons from Black Hawk County, Iowa who ingested varying amounts of nitrate via well water. Levels of NO3 in well water were linked to gastrointestinal complaints and bone/joint/muscle/nerve complaints (especially osteoarthritis). Levels of ingested NO3 positively correlated with IL-10 production ex vivo, and IL-10 production correlated with bone/joint complaints (Zeman et al., 2011).

This paper builds on that previous work, examining the effects of the ex vitro (in the lab) exposure of PBMCs to various levels of nitrate-nitrogen. PBMCs in this study were isolated from the blood of individuals with osteoarthritis and a control cohort. This study examined the impact of sodium nitrate exposures ex vivo (outside of the body), on PBMC cells from sodium nitrite exposure through a range of exposures representing values equal to or less than the regulatory level exposures in the US under the Safe Drinking Water Act, SDWA of 10 ppm Nitrate-N. Sodium nitrite served as a proxy for drinking water nitrate/nitrite exposure. PBMC were isolated from the whole blood of donors, grown in cell culture, and levels of IL-1, IL-6, IL-17, TNF-α, and IL-10 were determined simultaneously using Luminex multiplexing technology. Indicators of DNA damage to these critical immune system cells were studied as well. Indicators of induction pathway dephosphorylation (ataxia-telangiectasia mutated protein kinase, ATM) and single and double-stranded DNA breaks (histone H2A variant, PH2AX) were determined using a Muse flow cytometer with proprietary immune assay tags (Shrestha, Beltz & Zeman, 2021).

Method

Institutional Review Board (IRB), Participants, Blood Sample Collection, and Questionnaire

This study was approved by the IRB at the University of Northern Iowa (Protocol Number 14-0018). Participants were recruited as a convenience sample with advertisement across the University Campus and in Campus outpatient physical therapy clinics. Each participant consented to give their blood and fill out a short questionnaire survey that included information on demographics, general health related questions such as recent immunization, comorbidities, stressful life events (past 6 months), smoking and alcohol consumption history as well as information about arthritis symptoms and pain. Participants with reported autoimmune disease such as rheumatoid arthritis and psoriasis were excluded from participation. The screened participants were categorized in two distinct groups. Group 1 or Case Group were self-reported osteoarthritis sufferers and Group 2, or Control Group were participants without osteoarthritis complaints. Venous blood was collected from 15 participants from the case group and 16 from the control group. Overall, the participants' ages ranged from 40 to 65 years.

PBMC isolation, Nitrite Exposure, and Incubation

PBMCs composed primarily of T lymphocytes and monocytes, were isolated from the whole blood by standard Ficoll-Hypaque density gradient centrifugation (Kanof, Smith, & Zola, 1996). PBMC counts and viability were standardized with the initial concentration of cells being 4x106 cells/ml. Cell counts (concentrations) and viability were established using the Luminex MUSE Unit Cell Analyzer and Flow Cytometer gated at 98%. The MUSE unit was standardized and checked weekly when not in experimental use for instrument reliability and validity with a microbead standard, system check kit and a system check was also run prior to each experimental run.

The PBMCs of each of the participants were then resuspended in RPMI 1640 that contained 10% fetal bovine serum and100µl/ml penicillin/streptomycin. They were then placed into 96-well microliter tissue culture plates in triplicate. The PBMCs were exposed according to five different nitrite concentrations (sodium nitrite) at 0 (not exposed to nitrite solution) 1 mg/l (0.02 mM), 5 mg/l (0.10 mM), 10 mg/l (0.21 mM), and 15 mg/l (0.32 mM). The cells were cultured and dosed under two different conditions, stimulated and unstimulated. Under the stimulated condition, each cell-well was treated with 4 mg/ml of the stimulant phytohemagglutinin (Sigma Aldrich, Product # L1668) sourced from crude extracts of the red kidney bean Phaseolus vulgaris, while non-stimulated cells were incubated with the same volume of culture medium. All tissue culture plates were incubated at 35 o C with 5% CO2 acting as a buffer.

Determination of PBMC Numbers and Viability

Luminex Muse Counts and Viability DNA die binding reagent (sodium azide) allows the quantitative analysis of cell count and viability by preferentially binding to viable and non-viable cells based on the presence of DNA residues. The assay provides reliable results with cell concentrations in the range of $1x10^5$ to $1x10^7$ cells/ml. The cell samples for this research were used at a concentration of $4x10^6$ cells/ml. The analysis provides three results: viable cell count, total cell count, and percentage viability of the sample.

The sodium azide dye stains cells which have lost their membrane integrity, allowing the dye to stain the nucleus of dead and dying cells. The Muse unit scores viable cells as live cells that do not stain and non-viable, dead or dying cells that do stain. After 96 hours of incubation, PMBC was mixed with the Counts and Viability Reagent and then incubated for 5 minutes at room temperature. After incubation, the cells were analyzed with a gating set at the 98% threshold level to exclude debris. Finally, the counts and viability results were recorded and analyzed. A detailed methodology and test principle is provided in the Muse Count and Viability Kit User's Guide (Luminex Corporation Muse Counts and Viability Kit, 2020).

At this time a small aliquot of supernatant was pulled for cytokine analysis. Unstimulated PBMC do not produce detectable levels of cytokines, so they were not assayed; only stimulated cell supernatant was used for that purpose. The supernatant was then frozen at -40 C and later, following test scheduling, shipped overnight to the Case Western Reserve CTSC Bioanalyte Core Center packed in dry ice.

Determination of DNA Damage to PBMC

Muse DNA stress analysis involves the detection of Ataxia Telangiectasia (ATM) activation, a precursor to multiple phosphorylation induced factors, including the H2A.X family of histone proteins. Activation of ATM indicates phosphorylation linkage disruption while the presence and accumulation of ATM and H2A.X is indicative of double-stranded DNA breaks. The use of ATM and H2A.X specific binding antibodies allows for an analysis of the percentage of phosphorylation induction (ATM-activated cells), percentage of histone activated cells (H2A.X presence), and the percentage of DNA double-strand breakage (both ATM and H2A.X

expressing cells). This procedure followed the Muse Multi Color DNA Damage Kit User Guide to determine the DNA Damage among the PBMC. Cells were centrifuged at 300 g for 5 min, washed with phosphate-buffered saline, and treated with a permeabilization buffer for 10 minutes on ice, and washed again. Cells at a concentration of $2x10^5$, were exposed to a cocktail of assay buffer and antibody and incubated for 30 minutes in the dark. Cells were then resuspended in an assay buffer and analyzed using the DNA damage software profile on the MUSE unit (Luminex Corporation Muse Multi-Color DNA Damage, Methodology, 2020).

Determination of Cytokine Expression

Serum levels of the inflammatory cytokines IL-1β, TNF-α, IL-6, and IL-17 and the anti-inflammatory cytokine IL-10, were determined by the CTSC Bioanalyte Core Center of Case Western Reserve University, CWRU using the Luminex xMAP multiplexing ELISA system, which obtains data from multiple cytokines from each individual sample, then reads and analyzes those with the Luminex 200™ and xPONENT® software (Luminex Corporation, EMD Millipore) as described in (Almundarij et al., 2016). This methodology relies on the well-established ELISA method, grounded in the principle of antigen-antibody recognition.

Luminex uses a sandwich immunoassay wherein a capture antibody coats tiny polymer beads that are in suspension. These beads are coated with capture monoclonal antibodies specific to the cytokine(s) under investigation and are tagged individually with a unique fluorescent dye. The coated beads are incubated with supernatant and cytokine specific binding occurs. Lasers in the flow cytometer read, count, and collate the unique fluorescence signatures which are compared to a standard curve corresponding to the unique bead set/cytokine and reported out. Multiplexing equipment allows for multiple, different (up to 100) fluorescence signatures to be read and differentiated at the same time. Importantly, the data was analyzed in a blind manner as the research lab at CWRU did not have information about case/control or exposure status when running the analysis.

Quality Control, Data Management, and Analysis

The research protocol utilized required that a system check procedure be performed weekly while actively using the MUSE Unit. This procedure checked the reliability and accuracy of the Unit. In addition, the researcher also performed the system check prior to any experiments that included the Unit such as cell counts, viability, and DNA Damage. All reagents used for this research were stored at appropriate temperatures and followed the strict protocol provided by the manufacturer including adherence to expiration dates.

The data collected from the MUSE Unit were analyzed using JMP 15 statistical software from SAS institute. Statistical analysis included descriptive, bivariate and multivariate analysis including demographic descriptive data, bivariate fit and ANOVA correlational analysis with significance set at $p < 0.05$.

Results

Demographics

There were 31 total participants in the study representing 15 participants reporting osteoarthritis, (cases) and 16 participants reporting no osteoarthritis, (controls). The mean age of cases was 54.1 years with controls reporting 52.6 years. Most participants were female (77%). Life changing, stressful events (known to influence immune response) were reported by 4 total participants (2 cases and 2 controls) and a Chi-Square examining osteoarthritis status and life changing events was not significant with either the Pearson's or Likelihood Ratio test (p= 0.95). Six percent of the sample reported comorbidities and 55% of the sample reported regularly taking prescription medications, while 52% reported supplement use (see Table 1).

Table 1. Study Demographics

Demographics	Results
Age	Case: Mean (54.1 + 5.77) and Control: Mean (52.6 + 8.30)
Sex	Female (77%); Male (23%)
Life-Changing Events	Yes (13%); No (87%)
Comorbidities	Yes (6%); No (94%)
Medications	Yes (55%); No (29%); No Answer (16%); Mean (1.88 + 2); Range (0-6)
Supplement Use	Yes (52%); No (26%); No Answer (22%); Mean (1.8 + 1.68); Range (0-6)

Counts and Viability: Coefficient of Variation

The Coefficient of Variation (CV) in immunological toxicology studies examining lymphocyte counts is determined by counting the number of viable cells under various treatment/exposure conditions in both the antigenically stimulated and unstimulated states and applying the following formula:

cv = s/ns, wherein, cv= coefficient of variation, s=stimulated, and ns = non-stimulated state.

Thus, the CV is not an actual count of cells but a measure of the coefficient of change between non-stimulated cell growth and stimulated cell growth. In Figure 1, below, as the cohort of nitrite exposure increases, there is an attenuation of this cell coefficient with a significantly higher rate of cell proliferation at 1 ppm nitrate exposure (5.4761, p=0.0264) and a drop in proliferation at 5 ppm followed by a recovery at 10 and 15 ppm sodium nitrite

exposure but still exhibiting suppression of the coefficient compared to the lowest level of exposure (1 ppm) (see Table 2 for specific values).

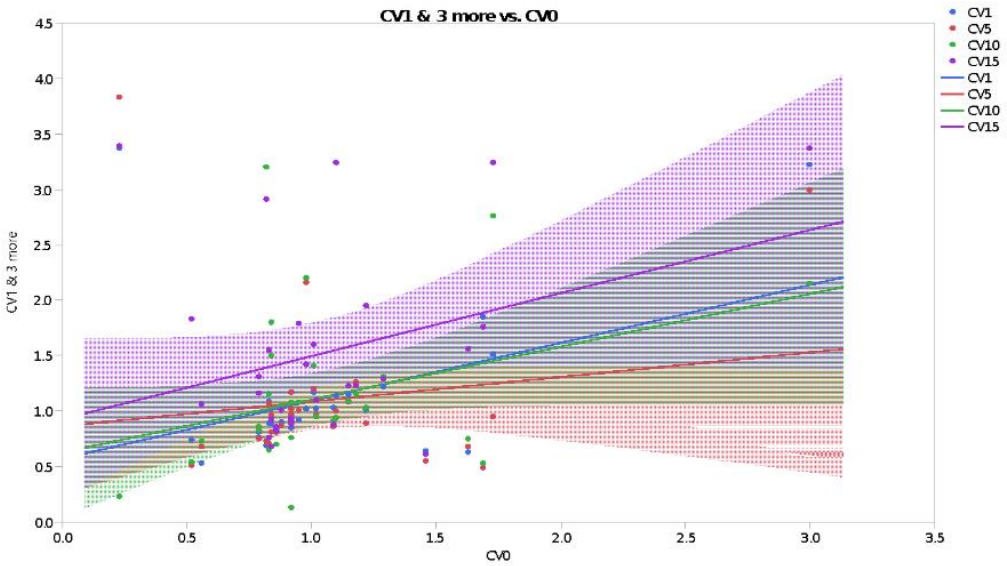

Figure 1. Coefficient of Variation across Nitrite Exposure Cohorts

Table 2. Specific CV Values across Exposure Cohorts, (N=31)

CV0	CV1	CV5	CV10	CV15
	5.4761, 0.0264*	0.6905, 0.4128	3.7801, 0.0616	3.4614 0.0730

Cytokine Expression

This study examined the proinflammatory cytokines IL-1β, IL-17, TNF-α, and IL-6. It also examined the anti-inflammatory cytokine IL-10. The proinflammatory cytokines IL-1β, IL-17, TNF-ȁ, and IL-6 all showed a strong response to increasing levels of nitrite exposure. In the case of IL-1β and IL-6 (bivariate fit, p=0.0001). Figure 2 below illustrates the cytokine response (in nanograms per liter, ug/l) based on the nitrate exposure cohort while Table 3 indicates the mean levels of cytokines expressed (ug/l) and the specific f-factors (weights) and p-values associated with each exposure cohort when compared with the control (no nitrite exposure).

IL-1β expression increased steadily and significantly in a dose-dependent manner until dropping significantly but remaining elevated above the control state. In all instances, the differences were statistically significant at p= <0.0001. For IL-6, there was a significant drop from the control state followed by a leveling off and significant increase at 10 ppm nitrite exposure and another significant decrease at 15 ppm (p = <0.0001). There was a major outlier in this group. Since the sample size was small (N=31), the outlier was excluded, and the bivariate fit ran again. The response with the strong or super-responder isolated showed a dose response

increase that was significant through each exposure cohort except for the highest exposure level of 15 ppm (p=<0.0006 -0.0001).

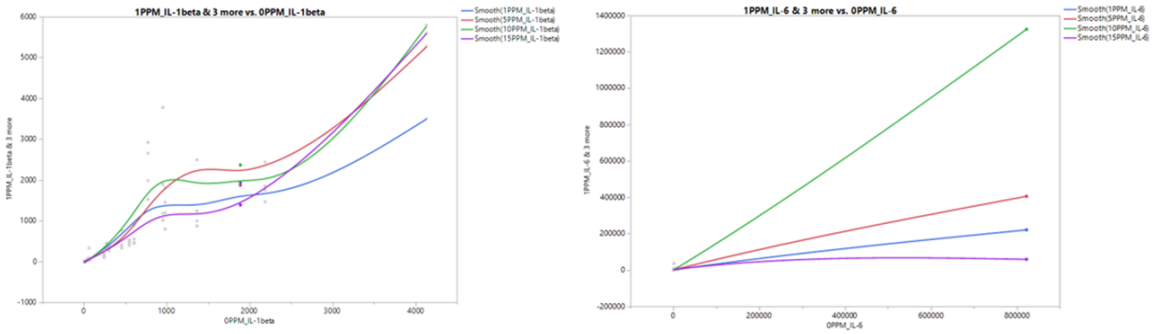

Figure 2. Cytokine Response IL-1 β, IL-6 at all Exposure Cohorts

Table 3. Mean Cytokine Expression (nanograms/liter) by Exposure Cohort, IL-1β, IL-6 and (fprob, P-Value, N =31)

0ppm IL-1ß	**1ppm IL-1ß**	**5ppm IL-1ß**	**10ppm IL-1ß**	**15ppm IL-1ß**
652.26 ug/l	688.18 (74.23, <0.0001*)	882.27 (234.01, <0.0001*)	938.90 (63.27, <0.0001*)	699.90 (223.91, <0.0001*)
0ppmIL-6	**1ppm IL-6**	**5ppm IL-6**	**10ppm IL-6**	**15ppm IL-6**
38,820.43 ug/l	11,746.1 (34231.82, <0.0001*)	11,746.1 (46713.00, <0.0001*)	65,137.28 (148168.10, <0.0001*)	5,798.02 (45.07, <0.0001*)
IL-6 Outlier Excluded 1,489.56 ug/l		1,881.45 (59.15, <0.0001*)	2,180.92 (17.25, 0.0006*)	3,266.61 (2.35, 0.1418)

The clear, statistically significant response pattern continued when examining TNF-α- and IL-17 across the exposure cohorts as can be seen in Figure 3 and Table 4 below with high significance levels reported when compared to control (p= <0.0001). It should be noted that there was a significant drop in TNF-α at 10 ppm nitrite exposure and a rebound at 15 ppm in this participant population. IL-17 expression initially increased at 1 ppm exposure and continued that trend at 5 ppm with a drop at 10-15 ppm nitrite (p = 0.0001).

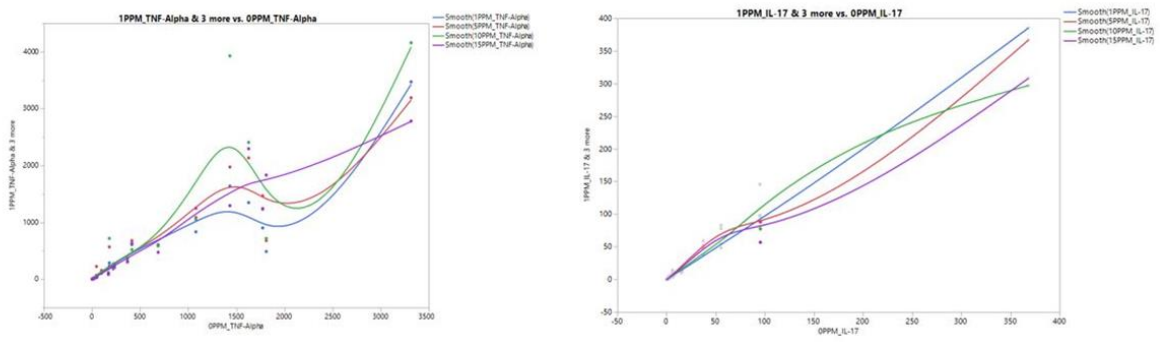

Figure 3. Cytokine Response TNF- α and IL-17 at all Exposure Cohorts

Table 4. Mean Cytokine Expression (nanograms/liter) by Exposure Cohort TNF-α, IL-17 and P-Value (N =31)

0PPMTNF-α	**1PPM TNF-α**	**5PPM TNF-α**	**10PPM TNF-α**	**15PPM TNF-α**
567.27 ug/l	499.40 (110.70, <0.0001*)	593.58 (135.74, <0.0001*)	721.03 (56.52, <0.0001*)	549.20 (293.36, <0.0001*)
0ppm IL-17	**1ppm IL-17**	**5ppm IL-17**	**10ppm IL-17**	**15ppm IL-17**
30.35 ug/l	32 (14787.69, <0.0001*)	31.57 (1996.89, <0.0001*)	29 (459.98, <0.0001*)	27.58 (975.24, <0.0001*)

For IL-10, which primarily acts to down regulate inflammation, there was an increase in expression across the 1 ppm and 10 ppm nitrite exposures, followed by an attenuation effect at 15 ppm. This is an interesting pattern that was seen with several of the pro-inflammatory cytokine's IL-1ß, IL-6 (particularly with outlier excluded), and IL-17, which is illustrated in Figure 4 and Table 5 below. In all cases, the response was statistically significant at p= 0.01 <0.0001.

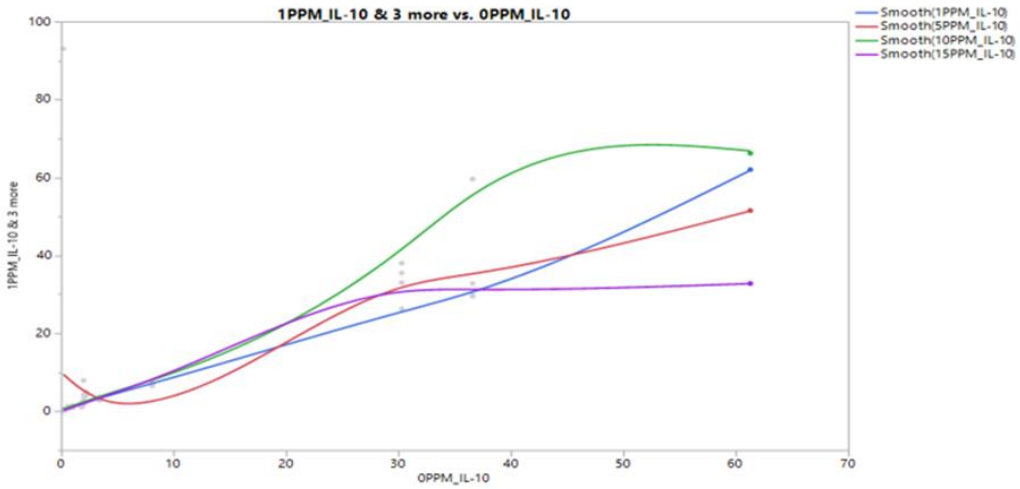

Figure 4. Cytokine Response IL-10 at all Exposure Cohorts

Table 5. Mean Cytokine Expression (ug/l) by Exposure IL-10 and P-Value (N =31)

0ppm IL-10	1ppm IL-10	5ppm IL-10	10ppm IL-10	15ppm IL-10
6.93 ug/l	6.86 (1685.421, <0.0001*)	11.23 (7.0497, 0.0152*)	9.20 (433.5097, <0.0001*)	5.76 (169.9070, <0.0001*)

DNA Damage

Markers of DNA damage, including the ATM, PH2AX, and DNA damage measures reported as percentage of cells exhibiting phosphorylation linkage disruption and histone damage indicative of DNA strand breakage. In the case of PH2AX, under both the non-stimulated and stimulated conditions, the percentage of cells expressing indications of phosphorylation disruption were elevated at increasing percentages at higher levels of exposure. In all instances, the percentage of elevated PH2AX values were statistically significant as shown in Figure 5 and Table 6.

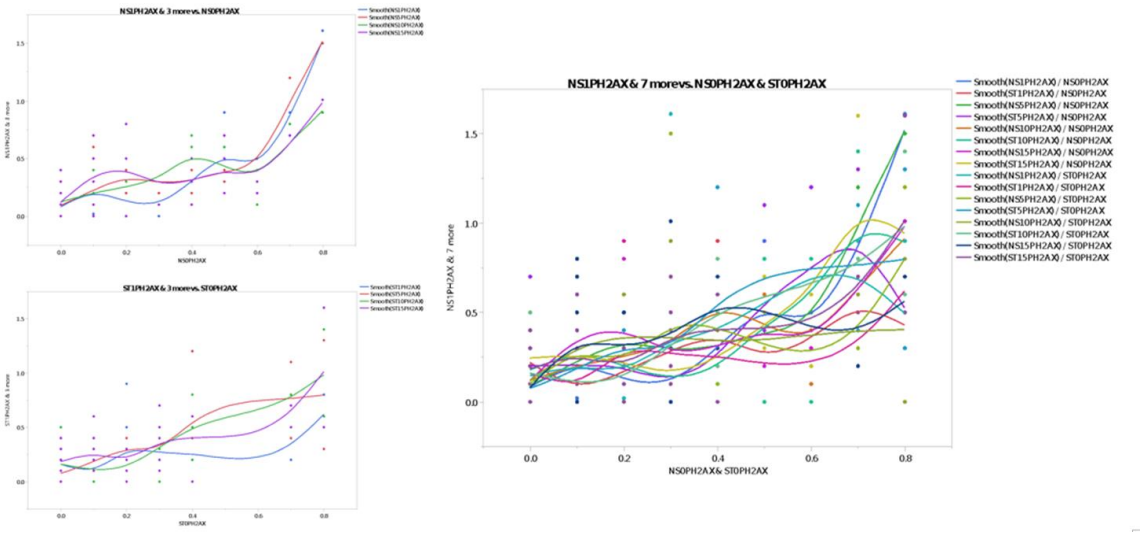

Figure 5. Non-Stimulated [NS], Stimulated [S], and NS to S PH2AX percentage at all Exposure cohorts

Table 6. Percentage Cells Indicating PH2AX Phosphorylation and Double Strand Breaks both Non-Stimulated and Stimulated (N =31)

Exposure Cohorts (Non-Stimulated)	1ppm- PH2AX	5ppm- PH2AX	10ppm- PH2AX	15ppm- PH2AX
0ppm-PH2AX 0.23%	0.27 (32.9644, <.0001*)	0.3 (31.8556, <.0001*)	0.28 (25.1676, <.0001*)	0.30 (10.075, 0.0035*)
Exposure Cohorts	**1ppm- PH2AX**	**5ppm- PH2AX**	**10ppm- PH2AX**	**15ppm- PH2AX**

(Stimulated)				
0ppm-PH2AX 0.23%	0.23 (7.5231, 0.0103*)	0.3 (22.1188, <.0001*)	0.29 (37.5187, <.0001*)	0.33 (20.6174, <.0001*)

ATM indicator percentages for the unstimulated state were not as responsive to nitrite exposure as in the stimulated state which after a slight drop at 1 ppm showed an increasing percentage of cells expressing ATM (p= <0.0001). Figure 6 and Table 7 provide a graphic and numerical data for percentage of cells expressing ATM.

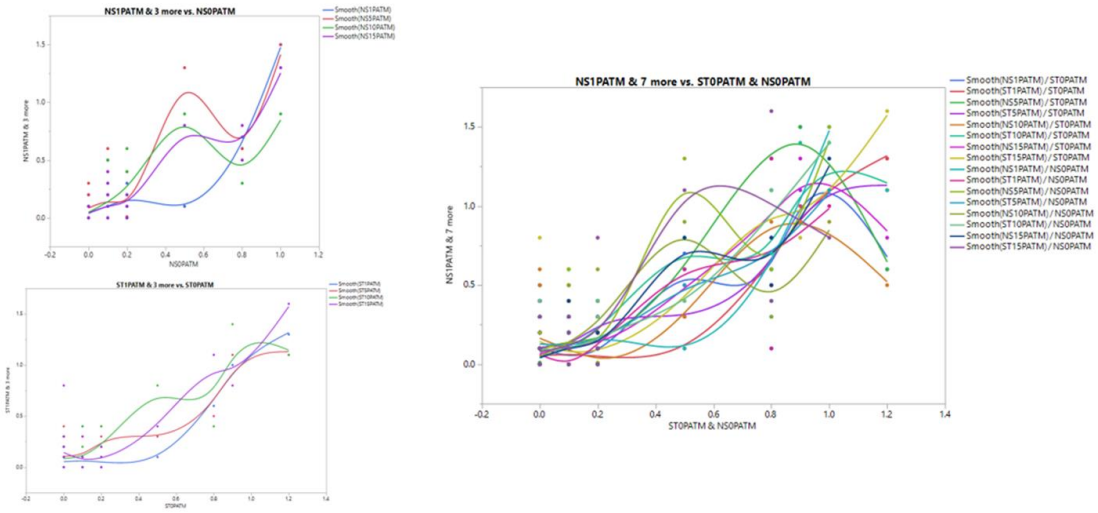

Figure 6. Non-Stimulated [NS], Stimulated [S], and NS to S pATM percentage at all Exposure cohorts

Table 7. Percentage of Cells Indicating pATM Expression, Non-Stimulated and Stimulated (N =31)

Exposure Cohorts (Non-Stimulated)	**1ppm- pATM**	**5ppm- pATM**	**10ppm- pATM**	**15ppm- pATM**
0ppm-pATM 0.17%	0.17, (71.9413, p=<0.0001*	0.23, (40.2961, p=<0.0001*)	0.20, (32.3667, p=<0.0001*)	0.18, (109.7469, p=<0.0001*)
Exposure Cohorts (Stimulated)	**1ppm- pATM**	**5ppm- pATM**	**10ppm- pATM**	**15ppm- pATM**
0ppm-pATM 0.16%	0.15, (96.8578, p=<0.0001*)	0.21, (91.9949, p=<0.0001*)	0.22, (71.6604, p=0.0001*)	0.23, (64.8207, p=<0.0001*)

As a final measure of impacts to DNA overall, both phosphorylation disruption and histone damage are considered as a cumulative percentage of DNA damage identified as “total DNA” in both the stimulated and unstimulated state. When all DNA damage indicators were considered as a progressive measure of DNA

disruption, a clear trend of increasing damage relative to increasing nitrite exposure becomes evident at the p= <0.0001 range of significance. Figure 7 and Table 8 below provide specific percentages, weights,and p-values.

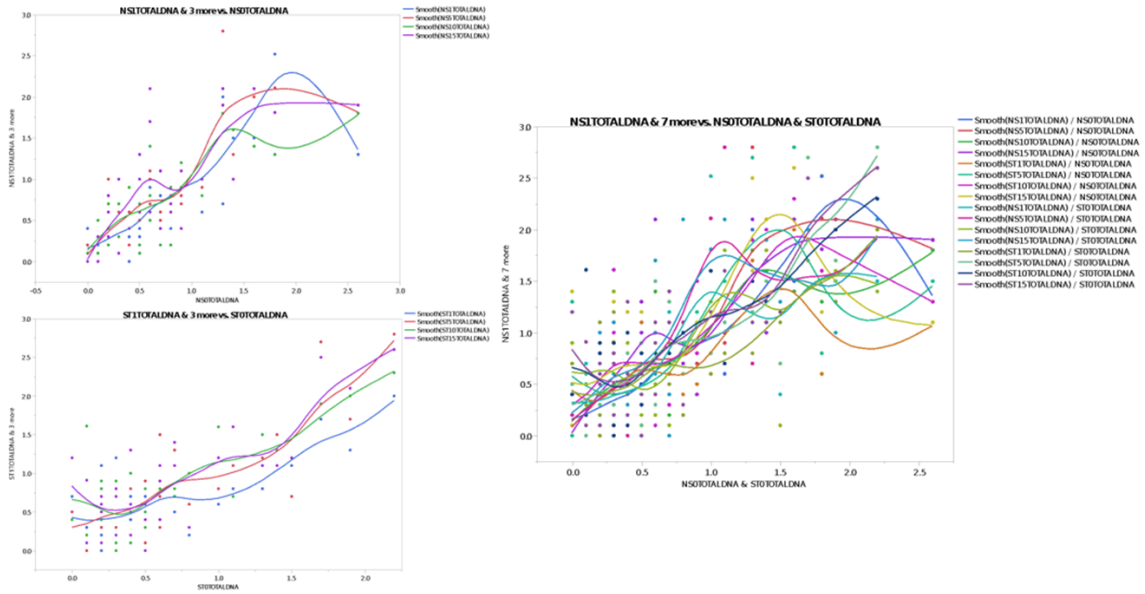

Figure 7. Non-Stimulated [NS], Stimulated [S], and NS to S Total DNA percentage at all Exposure cohorts

Table 8. Percentage of Cells Exhibiting Total DNA Damage, Non-Stimulated and Stimulated (N =31)

Exposure Cohorts (Non-Stimulated)	**1ppm- Total DNA**	**5ppm- Total DNA**	**10ppm- Total DNA**	**15ppm- Total DNA**
0ppm-Total DNA 0.7%	0.71 (42.0587, <.0001*)	0.83 (50.7601, <.0001*)	0.77 (43.4737, <.0001*)	0.89 (31.0690, <.0001*)
Exposure Cohorts (Stimulated)	**1ppm- Total DNA**	**5ppm- Total DNA**	**10ppm- Total DNA**	**15ppm- Total DNA**
0ppm-Total DNA 0.68%	0.68 (31.0014, <.0001*)	0.82 (55.0075, <.0001*)	0.85 (42.5398, <.0001*)	0.90 (42.9235, <.0001*)

Cytokine Expression and Various other Demographics and Health Factors

Finally, in regard to the questionnaire items for demographics and general health and wellbeing including age, gender, life-changing events, recent immunization, current smoking, osteoarthritis diagnosis, bone pain complaints, number of herbal supplements taken, and number of medications taken there was no statistically significant correlation effect on the levels of any tested cytokine, phosphorylation disruption nor double- or single-stranded DNA damage.

Discussion and Conclusions

NO and its metabolites nitrite and nitrate have complex relationships with human health. They help maintain homeostasis, but may also contribute to disease development. This research indicates that PBMC cells are responding with perturbations in inflammatory and antiinflammatory cytokine levels to nitrite exposures in vitro and that markers of DNA damage are exhibited by an increasing percentage of cells with increasing levels of nitrite exposure. Findings from this study provide points of discussion and on-going research for chronic health concerns regarding nitrate/nitrite exposures in drinking water. This is particularly important when nitrate/nitrite exposures are through drinking water and, thus, are unopposed by a complex food matrix, which includes a variety of antioxidant substances that tend to moderate nitrate/nitrite and its bioactive actions. Repeated studies have shown the ability of ingested nitrate to convert in the body to nitrite and nitric oxide, increasing the levels of these bioactive molecules. NO is produced from nitrate by nitric oxide synthase and this process is altered by cytokines from immune cells.

From the perspective of regulatory physiology, NO is a vasodilator, increasing blood flow to hypoxic regions and decreasing blood pressure (Halliwell & Gutteridge, 2007). It aids in the treatment of hypertension, coronary and peripheral artery disease, and heart failure (Peters et al 2003). NO is also a neurotransmitter, functions in signal transduction and immune system regulation, and alters protein functions (Hu & Zhu, 2014; Ghimire et al., 2017; Somasundaram et al., 2019). Alternatively NO is also playing a major role in inflammation and many chronic disease processes, iNOS is expressed by activated monocytes and macrophages, chondrocytes, synovial fibroblasts, and osteoblasts to produce high levels of NO. The proinflammatory cytokines TNF-α, IL-1, IL-6, and IL-17 as well as IL-10 stimulate iNOS activity (Nazleil et al., 2002; Vuoloteenaho et al., 2007). These cytokines, together with NO, play significant roles in encouraging the severity of various autoimmune and chronic disease conditions which are driven by inflammation, including such chronic diseases as rheumatoid arthritis (RA), osteoarthritis (OA), multiple sclerosis (MS), cirrhosis of the liver, fibrosis of the lung and kidney and numerous other conditions.

Pathophysiology of RA and OA: NO and Cytokines

NO and its metabolites have been linked to the pathophysiology of RA and OA (Grabowski et al., 1996; Onur et al. 2001; Wahl et al 2003). Significantly higher levels of NO are present in RA patients compared to controls. Additionally, higher levels of NO are found in patients with active disease compared to patients without active disease (Ali, 2014). In patients with active disease, NO levels are positively correlated with morning stiffness, arthritis, platelet count, C-reactive protein levels, and Disease Activity Score. NO levels correlate significantly with disease activity, inflammatory markers, and radiological joint status (Ali, 2014).

Cytokines examined in this study are central to inflammatory responses in OA and RA. Patients with RA and OA have synovial fluid and joints that contain high levels of inflammatory IL-1 and TNF-α (Guarner et al., 1993; Kirkham, 1991). IL-1, acting via NO production, decreases extracellular matrix synthesis, activates destructive metalloproteinases, inhibits synthesis of reparative collagen and proteoglycans, stimulates

chondrocyte death, and induces bone resorption (Grabowski et al., 1996; Armour et al., 2001). TNF-α increases cartilage degradation, bone resorption, and NO production (Halliwell & Gutteridge, 2007).

IL-6 also plays a role in the development and progression of RA in part by recruiting neutrophils and macrophages into inflammatory joint lesions (Narazaki, Tanaka, & Kishimoto, 2017). During RA, the presence of IL-6 in the joints triggers bone resorption by osteoclasts and aids in the production of several classes of antibodies, IgM and IgG, which serve as rheumatoid factors. OA and RA pathogenesis are associated with the presence of IL-1 and TNF-α. These cytokines induce production and secretion of NO and prostaglandin E2 (LeGrand, 2001). The latter is associated with inflammation and pain perception. IL-1β induces NO and E2 production to a greater degree than TNF-α and causes greater destruction of joints (LeGrand, 2001).

IL-17 and its receptors are found in many locations throughout the body, including in articular cartilage of synovial joints (Moseley et al 2003). This cytokine plays a role in the severity of both OA and RA (Miossec & Kolls, 2012). Serum IL-17 concentrations were significantly higher in patients with knee osteoarthritis than in controls. Among patients, synovial fluid, but not serum, levels of IL-17 increased significantly with Kellgren and Lawrence grade and was significantly correlated with the Lequesne index (r=0.6232) (Chen 2014). Menisci of both OA patients and controls constitutively produce NO and its production increases in the presence of IL-1β, TNFα, or IL-17. Furthermore, exposure to both IL-17 and TNF-α produced significantly increased NO production additively or synergistically (Legrand, 2001; Van Bezooijen et al., 2002). IL17 also reduces the synthesis of proteoglycan and collagen that are used in the repair of the affected joint. It also stimulates osteoclastic bone resorption (Van Bezooijen et al., 2002). One way in which IL-17 contributes to cartilage breakdown is by promoting the release of chemokines by chondrocytes (Honorati, 2019). Chemokines are immune messenger molecules that induce immune cells into specific areas. IL-17 promotes the release of the chemokine IL-8 by synovial fibroblasts and chondrocytes (Honorati, 2002). There is a genetic component to osteoarthritis which includes which IL-17 polymorphisms are expressed. Serum IL-17 levels significantly correlated with increased risk of OA in the knee joint (Bai et al., 2019).

When compared to IL-1β, IL-17 was more active on chondrocytes than on fibroblasts. In chondrocytes, the expression of IL-1β mRNA was enhanced by IL-17 and TNF-α. IL-17 could contribute to cartilage breakdown and synovial infiltration in OA by inducing both the release of chemokines by chondrocytes and synovial fibroblasts and production of IL-1β by chondrocytes (Honorati, 2002).

NO and Inflammatory Cytokines: Other Chronic Diseases

NO levels are increased in other chronic autoimmune/inflammatory diseases including inflammation-induced osteoporosis (Amour et al 2001), multiple sclerosis (Giovannoni et al., 1998; Nazleil et al., 2002), Crohn's disease (Rachmilewitz et al., 1995), Hashimoto's thyroiditis (Vural et al., 2009), lupus nephritis (Peters et al., 2003), and ulcerative colitis (Rachmilewitz et al., 1995). Plasma levels of these compounds correlate with renal fibrosis (Peters et al., 2003), diffuse pulmonary fibrosis (Hsu 2007), and primary biliary cirrhosis (Hokari,

2002). In addition to osteoarthritis and rheumatoid arthritis, some of the cytokines in this study are also associated with other chronic, inflammatory conditions. Excessive levels of IL-17 play a role in multiple sclerosis, psoriasis, and Crohn's disease (Capone, 2020; Leonardi, 2018). During multiple sclerosis, NO activates T lymphocytes and produces TNF-α and IL-1 which destroy and damage oligodendrocytes (Nazleil, 2002). Elevated NO levels during rheumatoid arthritis and systemic lupus erythematosus result from iNOS activation by IL-1 (Vural et al., 2009). During cirrhosis of the liver and chronic hepatitis, increased nitrite and nitrate levels correlate with TNF-α levels (Guarner, 1993; Tankurt et al., 1998). NO metabolites also play roles in fibrotic diseases such as diffuse pulmonary fibrosis and experimental lupus nephritis (Peters et al., 2003).

IL-10 produced by Tregs downregulates excessive immune responses, protecting against autoimmune and inflammatory conditions. Tregs are dysfunctional in multiple sclerosis, Type I diabetes, and rheumatoid arthritis (Guarner et al., 1993). IL-10 inhibits generation of proinflammatory TNF-α and IL-1 (Vural et al., 2009). This study found that increasing nitrate exposure decreased serum IL-10 levels and may, thus, be responsible for the increased levels of TNF-α and IL-1.

IL-17 is being increasingly found to play a major role in the development of many acute and chronic disease conditions, partially since it induces production of other inflammatory mediators, including TNF-α, IL-1, IL-6, and NO (Lubberts, 2005). IL-17 is produced by both memory T helper cells as well as T killer cells (Chen, 2014). IL17 induces chondrocytes and fibroblasts to produce and release several other pro-inflammatory cytokines, including TNF-α, IL-lβ, and IL-6 as well as production of NO and collagenases that destroy cartilage (Southam, 20006; Honarati et al., 2002; LeGrand et al., 2001; Attur et al., 1997). The IL-17-mediated increase in NO production in OA cartilage occurs in the absence of IL-lβ signaling (Attur et al., 1997). IL-17 also increased proliferation of T cells stimulated by low levels of phytohemagglutinin (Broxmeyer, 1996, Zeman et al., 2011).

During another chronic disease condition, type II diabetes cardiomyopathy, levels of IL-10 in the heart are decreased (Azizian, 2018), while those of TNF-α and IL-6 increased, as well as reactive oxygen species (ROS), inflammatory factors, and the DNA damage marker 8-oxo-7,8-dihydroguanidine (8oxoG) (Azizian, 2018; Barouch, 2006). 8-OxoG is a common mutation formed after exposure of DNA to reactive oxygen species in a manner that results in guanine to thymine and cytosine to adenine substitutions

Atherosclerosis is an inflammatory chronic disease of the walls of arteries. The immunoinflammatory response is regulated by the anti-inflammatory cytokines IL-10 and transforming growth factor-β (TGF-β) (Tedgui, 2006). Proinflammatory cytokines expressed in atherosclerotic plaques, including TNF-α, IL-1, and IL-6, are present in the atherosclerotic plaques, as well as the regulatory cytokines IL-10 and TGF-β. TNF-α and IL-1 induce the production of toxic ROS, including superoxide, hydrogen peroxide, and the hydroxyl radical (Tedgui, 2006).

DNA Damage

The increased percentage of cells exhibiting PH2AX when exposed to nitrite in the treatment cohorts is an early marker of DNA double-strand breaks. Sustained expression of pH2AX may lead to irreparable DNA damage and cell death (Wilson 2012). Type 1 diabetes is associated with a higher level of DNA strand breaks in leukocytes and is negatively associated with serum nitrite concentration (Rostoka, 2021). A variety of cancers have been associated with DNA damage.

Summary

This study illustrated:

- IL-1, IL-6, IL-17, TNF-α, and IL-10 cytokine expression in the majority of exposure cohorts fluctuated in a statistically significant fashion, often in a directly proportional manner, especially in cohorts of 1-10 ppm nitrite exposure,
- DNA damage as indicated by the percentages of cells exhibiting PH2AX, ATM, DNA [histone expression] increase in response to increases in nitrite exposure,
- Increases in cytokine expression and DNA damage were seen irrespective of demographic characteristic or health status.

This was a small, in vitro study but suggests the need for additional work, especially in larger more diverse populations to understand the manner in which genetic diversity interacts with responses to environmental exposures and to understand the role that nitrate/nitrite exposures in drinking water play in chronic disease processes, wherein inflammation plays a primary role in severity of the disease process and in flares of severe episodes. These findings are particularly compelling when considering the number of epidemiological studies that have tied higher ingestion of nitrates in drinking water to various cancers and chronic inflammatory diseases.

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Applied Artificial Intelligence: Teaching Information Technology Students How to Utilize Commercially Available AI Tools

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Abstract: When one thinks of artificial intelligence, they often associate the field with computer science, data science, machine learning, and deep learning. Those are among the technical disciplines and research areas that continue to push the frontier of what is capable with artificial intelligence. But as work continues in the effort to develop new AI tools and capabilities, there is a growing suite of existing AI tools, commercially available, that can directly impact the operations of organizations today. These tools can and should be part of an Information Technology strategy that supports a company's mission. To help Information Technology graduates be better prepared with the knowledge of currently available AI tools, a course was developed that covered the history of AI, the ongoing direction of the field, and the more common categories of available, applied AI tools. This paper will describe the design of this course, its outcomes, and its reception by IT students.

Keywords: Artificial intelligence, Information systems, University curriculum, Commercially available tools

Introduction

For decades, there have been conversations about artificial intelligence (AI) and the many different scenarios that might play out if the vision of a thinking machine were ever to be achieved. Mitchell (2019) summarized the opposing sides of the AI argument. There are those who wholeheartedly embrace the advances made in the field and then there are those who don't believe thinking machines are possible. There are also those who see the pursuit of AI as an existential threat to our current way of life. While the researchers and ethicists debate the future of AI, there is no doubt that the field has already had a significant impact on how many organizations operate.

There are currently many types of AI tools that are commercially available and are being used to further organizational goals. Rouhiainen (2020) provides an overview of the impacts that AI is having on business, communications, the job market, travel, and a host of other domains where these technologies are currently being employed. These relatively new tools are being refined yearly and they require trained professionals to understand, implement, and support them. Often the success of AI tools is determined by how they are initially set up. Poorly understood or implemented AI will likely be a disappointment as it fails to realize the promises

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that drove its acquisition.

Many organizations rely on their information technology (IT) departments to craft an IT strategy that is aligned with and in support of the organization's overall strategy. Increasingly those IT strategies are including AI solutions. But how does an organization ensure that its IT people, who may be skilled in more common IT tools, are also skilled in AI tools?

To that end, an "AI Tools for IT Managers" course was drafted and offered to undergraduates as part of an information technology specialization. The class focused, primarily, on existing technologies that IT managers could encounter or consider in support of an organization's IT strategy. It was helpful to have the background to see where AI started, its initial promises, and its current state. This helped students understand the significance of the products we have today and how useful they might, or might not, be. But the majority of this class was mostly a survey of the current state of AI as it relates to organizational needs and IT solutions.

The original outcomes of the course were:

1. Describe the history of artificial intelligence (AI) and its evolution.
2. Describe classes of common AI applications that are commercially available for use in IT.
3. Evaluate the ways in which available AI tools can help organizations achieve their IT objectives.
4. Evaluate past and current ethical considerations regarding the use of AI technologies.

As university IT programs prepare the next generation of IT professionals, the curriculum needs to adapt in order to keep up with developments in the commercial space. New IT professionals need to be fully prepared to implement and support cutting-edge technologies and many of those will be based on AI tools. The remainder of this paper will describe how the AI Tools for IT Managers class was developed and taught and how it served to ensure the proper preparation of IT graduates regarding practical AI.

Outline of the Course

Offered in a ten-week quarter, the course consisted of ten modules. The early modules helped define AI and provided some historical background of the field. Each of the later modules focused on a class of commercially available AI tools or technologies. Students engaged in group discussions and completed individual assignments intended to meet the course's outcomes and ensure that they had a working knowledge of these tools and concepts. Most modules introduced two new topics; one had to do with commercially available tools and the other had to do with AI technologies. The layout of the course is shown in table 1 below.

Textbook

Fining a single textbook to cover both commercially available tools and provide a higher-level look at the

technologies that made them run proved to be a difficult task. The books were either too technical or spent too much time on the long history of the field. In the end, two textbooks were selected. The first was Rouhianinen's *Artificial Intelligence: 101 Things You Must Know Today About Our Future*. This book provides a good overview of common AI tools and their impact on various sectors. It does not, however, spend too much time reviewing the technologies that make AI work. So, a second text was added which was Taulli's *Artificial Intelligence Basics: A Non-technical Introduction*. This text provides a high-level introduction to topics such as machine learning, deep learning robotics, and natural language processing. The combination of the two texts was a good fit for the focus of the course and it gave students a more robust introduction to the current state of commercially available AI.

Table 1. Outline of the AI Course

Module	Key Topics Covered
1	Foundations and definitions of AI & Examples of AI
2	History of AI and thinking machines & AI winter
3	Industry impacts & Big data
4	Business processes, Machine learning, & Concerns with AI
5	Chatbots & Deep learning
6	Workplace impacts & Natural Language Processing
7	Autonomous Transportation
8	Robots & Ethical considerations of robotics
9	AI's Major Players
10	Final culminating project

AI Tools

As indicated above, the early part of the class focused on the history, evolving definition, and likely trajectory of AI. To appreciate where the field is today, it was important for students to understand the long path AI has been on since the early days of computer technology. Considering some of the topics dating all the way back to the 1950s, students were asked to share their thoughts about the booms and busts of AI and what it means to have a thinking machine. The course also introduced students to the importance and applicability of big data sets in developing and operating AI tools. Additionally, the topics of machine learning and deep learning and their relevance to AI were covered, at a high level, just so students were familiar with them. Once these foundational topics were sufficiently covered, it was time to look at categories of AI tools.

Chatbots

The first category of AI tools that the students looked at was chatbots. A chatbot is basically a robot that does online chatting. They have had a big impact in the area of customer services and satisfaction, although the

satisfaction provided by certain chatbots is often suspect.

Customers interact with chatbots when seeking assistance, shopping, or in a number of other applications. The idea is to provide a customer with the option of asking the chatbot questions that the chatbot is programmed to answer. In truth, a chatbot is not much different than a search engine. It reads a question or request, parses the words that the users have typed, identifies the most important ones, and then searches through a list of the best possible responses to serve back. It repeats this process over and over again until the user's question is answered, or it can direct the user to another person or resource that will address the need. Chatbots can come in different levels of sophistication. The least technical can struggle with user requests, or they simply return a document for the user to read that may or may not be relevant. The best ones have levels of sophistication that make it feel like the chatbot is more like a live human.

The impact of chatbots has been huge. For decades, companies have spent a good deal of money and time on customer service employees and salespeople who spend a good amount of their time doing routine and repeatable tasks. All of that can be shifted to chatbots which require a one-time upfront fee and take the place of expensive employees. Plus, there is no limit to the availability of chatbots. Unlike employees, companies can make them available 7/24/365.

In this part of the AI class, students were asked to examine the functionality of chatbots and consider the differences between effective and poor implementations of these tools. They were also assigned to read about how the level of upfront effort required to make a chatbot effective and the best practices for their use. They were then asked to find three chatbots online, spend time interacting with each, and provide a qualitative assessment of the features that they felt were most and least effective.

Natural Language Processing

Anyone who has spent time on today's more common tools that employ natural language processing (NLP) can attest to the fact that we have a long way to go in this area of AI. Language is volatile; it has different sounds, relies on context, and changes with time. Add that to the fact that language has homonyms, slang, and accents and it is clear why so many problems remain with NLP. Yet, even with its challenges, there have been great advances with NLP and many tools have been developed that utilize it. NLP can be used to record conversations, create content for consumers and websites, and it is the backbone of personal assistants. Its significance as a key part of an IT strategy will only increase in the coming years as the technology improves.

In this part of the class, students are asked to consider the many applications of NLP. But they also engage in a discussion of their own experiences with NLP where they are asked to discuss some of the challenges that still remain with this technology.

Autonomous Transportation

Autonomous transportation (or self-driving vehicles) is one topic that is covered quite a bit in the media mostly through stories of self-driving cars. But autonomous transportation covers much more than cars. The potential impact of this branch of AI on many different organizations is likely to be substantial so this was a significant part of the AI course.

A long-standing problem that organizations have had to tackle is how to get things to different places. At each step in the supply chain, there are considerations of logistics - how do we get things where they need to be. And with a global economy, that becomes even more complex. Today, products are shipped from vendors directly to people's homes. That could involve cargo ships or planes, and semi-trucks to receive the cargo and move it to warehouses where it is picked up by a shipping company, sorted, and then delivered. And customers are demanding - they want what they want when they want it.

The logistics of a supply chain cost a lot in terms of time and money. And there is great pressure to reduce both. Amazon is considering using drones to deliver packages. We are hearing of automation in the cargo and sorting process and self-driving cars may take over services such as taxis, Lyft, Uber, and food/grocery deliveries. We are already looking at autonomous transportation technology that can move people from one location to another in the forms of cars, buses, trains, and even planes.

From an IT perspective, students will need to consider the impacts of the use of autonomous transportation technologies. Students need to fully consider how these technologies are likely to impact all parts of an organization. This is one area of the AI course where students spent a significant amount of time. They were asked to share concerns about self-driving cars and then prepared a paper that addressed the viability, usefulness, and utility of autonomous transportation.

Robotics

Robots are often portrayed, in movies and on television, as sentient beings with human qualities. Those stories have largely been fantasies, but they do address some of the concerns that are associated with the development and use of robots.

Today's robotics reality is different. Robots are workhorses with intelligent technology that allows them to sense and adjust to their environments. However, as technology improves, we can expect that efforts will be made to expand the capabilities of robots.

Current robots are rules-based meaning they only do what they are programmed to do but there are development efforts in robotics that are driven by AI. These efforts have implications for the future capabilities and adoption

of these tools. Increasingly, there will be logistic, ethical, technological, and human questions associated with the use and development of robot technology.

Today's IT students need to concern themselves with only the workhorses. They need to consider the complexity of implementation and, given the cost of many robotic tools, there needs to be some consideration given to their financial viability as well. When looking to robotics as part of an overall IT strategy, students need to consider questions of justification, implementation, support, and utility. There are also the concerns of worker displacement and retraining, which is not necessarily an IT function but is something that could impact current and future IT professionals. These varied questions are all addressed in the robotics section of the AI course as students review the ethical considerations of robot technology and the rules of robotics.

Final Topics and Project

As the course begins to wind down, students shift their focus from categories of AI tools to the big players in the field who are responsible for much of the research, development, and commercial distribution of AI. The non-exhaustive list of big players includes Google, Facebook, Amazon, Microsoft, IBM, Apple, Nvidia, Alibaba, Baidu, and Tencent. While learning about the products and accomplishments of these big players, students are also reviewing the process of implementing AI in their own companies. Taulli (2019) dedicates an entire chapter of his book to implementing AI projects. His steps include identifying a need, assembling the right team, selecting the right tools and platforms, creating the AI model, and deploying the solution. In this part of the course, students get an opportunity to contrast well-funded AI research and development efforts against the types of smaller projects they are more likely to encounter as they deploy tools in their own companies.

Learning how to implement AI tools is good preparation for the course's final project which is a case study. Students are presented with a scenario that describes a global company for which they must propose a 5-year IT strategy. They are required to include AI tools as part of this strategy and come up with a plan that positions the company well against its competitors and reduces the cost of producing, selling, and servicing products. Students are to prepare an 8-10 slide presentation, with detailed notes, that could be delivered to the senior leaders of the company that is described in the scenario. In their presentations, they are to have selected a suite of integrated tools and they must indicate what has been proposed, why it was proposed and the impacts it will have on costs and competitors.

Conclusion

To date, this course has been run only once but students' response to it was very positive. The level of interaction in the discussions was very high, especially on questions where students were asked to share their opinions about the ethical nature of AI tools and development. Students were also asked if they had enough confidence in autonomous transportation to ride in a driverless car and this was another very lively discussion.

A series of weekly assignments, combined with five projects prepared students to be successful on the final project. In short, the work of the students demonstrated a high level of competence in the four course outcomes. The student evaluations were another indicator of positive response with students rating the course at 4.5 / 5.0 overall and at 4.62 / 5.0 specific to the way the course was taught.

This will be a course that will need to be updated frequently as AI technologies evolve. The questions of ethics and feasibility will persist as they have for decades and they may be shaped by technical developments in ways we cannot predict. But AI is here to stay, and commercial offerings will only expand. Educating IT professionals in the use of these tools should be the concern of all university-level IT preparation programs.

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Proposal for the Development of an Intuition Ecosystem Model Validation for Enhancing General Decision Making -IEM Validation

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Abstract: This opinion paper presents an INTUITION ECOSYSTEM MODEL -IEM, Figure a)- *and proposes, to whom it may concern, a general guide for developing a Validation Process for this IEM*, an innovative proposal seeking to enhance Intuition in general decision-making. The general road map presented in this opinion paper gives clues/hints to constructing the IEM Validation Process -a set of questionings for the MODE from Figure a). Developing this IEM Validation Process is a challenging and interdisciplinary process involving understanding human beings' cognition, pattern recognition, cognitive model, and decision making. In this opinion paper, we present a general road map for the Validation of the IEM -Figure a)-. No one has even supposed to develop a VALIDATION PROCESS for the MODEL presented in Figure a). We invite those *concerned with the issue of Intuition to work on the Validation Process* aiming for its use in general decision-making.

Keywords: Intuition decision making, Intuition ecosystem model, Validation model, Cognitive units, Set of questioning for validation model

Introduction

We present a MODEL proposal for an INTUITION ECOSYSTEM MODEL -IEM, Figure a) and propose the development of a VALIDATION PROCESS for this IEM. The development of this IEM Validation Process is an innovative proposal seeking to enhance Intuition in general decision-making. In this paper, *we present a general road map for developing this IEM Validation Process and call interested researchers to construct this Validation Process for the IEM, Figure a).* Developing this IEM Validation Process is a challenging and interdisciplinary issue involving understanding human beings' cognition, pattern recognition, cognitive model, and decision making.

THE INTUITION ECOSYSTEM MODEL -IEM

The Intuition Ecosystem Model -IEM- an adaptation from figure 1, reference Balloni & Feldman [01], is presented in Figure a):

Balloni, A.J. (2022). Proposal for the Development of an Intuition Ecosystem Model Validation for Enhancing General Decision Making -Intuition Ecosystem Model, IEM- Validation. In C.L. Zeman & H. Akcay (Eds.), *Proceedings of ILSET 2022-- International Conference on Life Sciences, Engineering and Technology* (pp. 29-42), Los Angeles, USA. ISTES Organization.

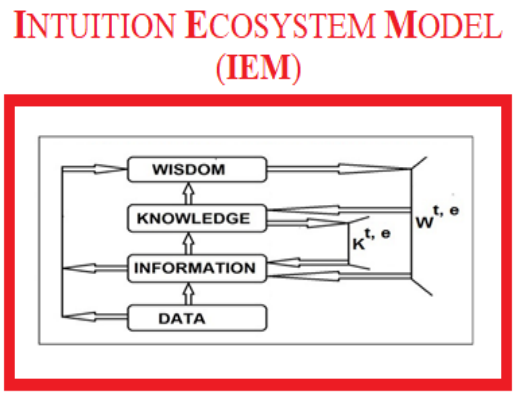

Figure a). Represents the Intuition Ecosystem Model -IEM-, composed of the same cognitive unites -D: Data, I: Information, K: Knowledge -t: tacit and e: explicit-, W: Wisdom -t: tacit and e: explicit-, *a Corollary Proven as published by reference [01]*. In the IEM, we propose to construct for each cognitive unit DIKW, a set of questions that should provide the intuitive solution we aim for when answered. This opinion paper describes a general road map -clues- towards constructing the Validation Process for the IEM. *We call interested researchers to construct this Validation Process for the IEM* and rescue intuition decision-makers by including Intuition in our day-to-day decision-making processes. *APPENDIX 1 presented a detailed description of each Cognitive Unit -D, I, K, W-.*

The main concern of this opinion paper is to address the correct set of questioning -IEM VALIDATION PROCESS-to each cognitive unit from the IEM, Figure a). The proposal is to guide to whom it may concern the development of a VALIDATION PROCESS FOR THIS IEM. Papers study should guide us to find a set of questions -the Validation process-regarding each cognitive unit presented in Figure a). An integrated analysis of these answers guides us to intuition decision-making. Finally, the method -the set of questioning- must be presented to several testers -from academics, businesses, etc.- which will apply the process according to their needs and interests. There are no papers addressing this interdisciplinary research topic with a gamma of practical applications and an enhancer for Research & Economics.

The bottleneck of this work is the Intuition Ecosystem Model Validation -IEM-. This Validation process must be guaranteed by the best set of questions. We invite those interested in the issue to hands-on and develop the validation process for the IEM, Figure a).

At last, we would like to quote:

a) - Reinhard Selten [02] regarding the importance of this Research: "…*the most efficient way to improve the decision-making process is improving the Intuition. The people who come with a great repertoire of simple scenarios of Management or games and their respective analyzes have more accurate Intuition and do not forget easily of important aspects that should be considered in the decision about a problem*…". And,

b) - Guido Schmidt [03] "*Science is good at describing systems, but science is not good at providing answers to how to manage these systems. And that is what science needs to get better at*".

Our Intuition Ecosystem Model Validation intends to provide answers through a set of questions!

State of the Art

After researching for a couple of years on the issue of Intuition, we realize that the development of the Intuition Ecosystem Model -Figure a)- is original and innovative. Besides this, one of the importance of the IEM is its applications for economic, social issues, and sustainability concerns. The Management of the economy reflects a social value-adding -the multi/interdisciplinary aspects of the IEM development. Regarding the importance of an IEM development, we have the "goals" listed in the "Environmental sustainability for the Sustainable Development," [04] European Union 2030 Agenda, as well the United Nations Division for Sustainable Development Goals, "Department of Economic and Social Affairs [05]. These goals support thematic issues, such as water, energy, science & technology, aiming to implement the UN system-wide 2030 Agenda. We may state from all the above that the IEM -Figure a)- reflects State-of-the-art Research.

Therefore, according to our conception, the IEM- as presented in Figure a) is a first endeavor and innovation toward general Management, Public Policy, and true Democracy. This paper provides evidence of how the IEM will become a "state of the art model," i.e., how it relates to the top research in the field and how it will move a step forward. Finally, as far as we know, based on the screening process of reference searching, no one has worked in such a challenging model development as proposed in Figure a). Nevertheless, no one has even thought about developing an *IEM VALIDATION PROCESS* regarding each cognitive unit from the IEM, Figure a), which is the main proposal of this opinion paper. *Finally, APPENDIX 1 presents a detailed description of the cognitive units D, I, K, and W.*

Research Objectives

The DEVELOPMENT of the INTUITION ECOSYSTEM MODEL VALIDATION -IEM MODEL VALIDATION- must guide decision-making toward constructing a better and more prosperous society. A multidisciplinary approach may be vital in several issues regarding decision-making, such as research & innovation management, education, strategic & systemic innovation, and business strategy.

Objectives

What Researchers interested in this opinion paper could do to find the best set of questioning for the validation model, i.e., -IEM MODEL VALIDATION-:

a) - Elaboration of a systematic literature review –deep and wide-on intuitions publications.

b) - Identifying the paper where our creative process may act in connection with the construction of the questionings for the IEM Validation -Figure a)-.

c) - Identifying the different scopes regarding the intuition application or consideration -analytics processes towards the correct and assertive construct of validation questionings-.

d) - Researching the role of different technologies regarding the intuition perspective and correlation with the questioning's creation.

e) - The final goal of this Research is to develop the validation process for the IEM. As described in this opinion paper, this Validation is composed of a set of questions constructed for each cognitive unit D, I, K, W -Figure a)-. APPENDIX 1 describes the mean of each cognitive unit.

According to Katry & Ng [06], regarding the validation process, "*Field research in applied management settings is quite spars; field research is virtually non-existent*." Our premise is that the Intuition Ecosystem Model will be the most decisive Model ever presented and applied to enhance general decision-making. Based on the Intuition Ecosystem Model -Figure a)-, we may reframe our Mindset throughout the IEM Validation Processes -the set of questioning we are looking for. The following Mindset is a must to construct the set of questions: to understand the critical aspects of your market business or research -its local or global trends or patterns... This Mindset, PLUS understanding the meaning of the MODEL, is central to finding out each question for each Cognitive Unit from Figure a). The paper published in the proceeding of this congress presents a detailed description of each Cognitive Unit

e.1 PRELIMINARY Validation Model Proposal.

Once the IEM Validation set of questioning is ready, it should be presented to scientists and entrepreneurs, asking them to apply the method according to their needs and interests. To implement the validation model, researchers should consider a sample of about 50 testers -it depends on the analyses from the readers of this opinion paper so that it could be more or less than 50 testers-. These testers should inform the percentage regarding success or failure using the IEM Validation process for decision-making. The Testers who apply the IEM Validation -scientists or entrepreneurs- should be looking for and at the best decision-making. Therefore, when applying the Intuition Ecosystem Model Validation, the TESTERS should be with the following Mindset or compliance:

a) - the Validation of the IEM works better if the decision-making is hard-hitting or challenging to find;

b) - the Tester should not be in "blue sky behavior" or easy decision making; otherwise, the IEM Validation could not "answer" to the true core of the issue. According to Meindertsma [07, "*…in uncertain situations, we become more confused in our choices. But this also leads to exploration, which helps us adapt to a new environment*…". An example of the *new environment* could be the implementation of the decision-making instrument -the set of questions.

- In short, the Tester should be passing through stress or forecasting stress -any kind-to use this decision-making instrument, the IEM Validation Tool. They also must know the meaning of each cognitive unit -see APPENDIX 1 definitions for D, I, K, and W**.**
- Note: this is an opinion paper, and, for sure, any Researcher may find a better way to get to the best set of questioning for the IEM Validation Process -Figure a).
- Section 2. -Effectiveness of the proposal for developing the validation tools -IEM Validation- & Risk Management-. Table 1 of this section presents the Risks a researcher could face when constructing the IEM VALIDATION MODEL -the set of questions-.

Research Methodology

Description of the Methodology aiming to create the best IEM Validation.

Research Questions

Before presenting the Methodology, we proposed the following questions as an initial guideline for the Research and the best finding of the IEM Validation Questioning**:**

a) - Which formal, non-formal, and informal practices of intuition utilization contribute to decision-making in organizations? What is the role of Intuition in decision-making?

b) - How is the intuition process triggered and implemented in an organization? Could the IEM -Figure a), presents an effective decision-making tool when integrating the results of all answers from the set of questionings developed for the Validation Process?

c) - Are there other models or frameworks which have considered the application of Intuition towards organizational decision-making?

d) - Is there any Intuition model developed for managerial decision-making -not found yet, up to this date-? Or is there any field research on such issues?

Research Methods towards the findings of the best set of questioning for the IEM Validation

a) -To do an extensive literature review regarding the utilization of Intuition by organizations. This an extensive review of literature will pass from a screening process, selecting specific papers with the most-cited authors and articles. Here we have a creative process towards creating our set of questions for our Intuition Ecosystem Model. By analyzing as many references as possible, a Researcher may be guided -*during the dynamic reading process and interactions among papers review, analysis, and summary*- toward drawing up the base set of questioning for the Intuition Ecosystem Model Validation-. The Researcher must always be available for searching further references;

b) -Section 1.3.4 describes the Validation process of the Intuition Ecosystem Model. However, the construction of the *preliminary validation process* follows the procedure as described in Research Objectives, section 1.2, item e)/e1.

The Intuition Ecosystem Model Validation

After a literature review and analysis & paper summary, we should be able to draw up the base set of questioning for each Cognitive Unite for the Intuition Ecosystem Model Validation -see also Research Methods, section 1.3.2, item b-. The review, analysis, and summary of papers is a creative process, a dynamic spiral work of "stop and go" –analyses, i.e., study the paper –look at what the paper offers- create questions, and do new research in the literature for each question you have made. The dynamic spiral of the "stop and go" procedure should continue

until the *Researcher finds a self-consistent set of questions for each cognitive unit DIKW -Figure a)-*. APPENDIX 1 presents the definitions for D, I, K, and W.

The Validation of the Intuition Ecosystem Model

The Validation Process of our IEM -Figure a)- must follow the procedure described in this opinion paper - section 1.2, item e)/e1-. However, the Researcher interested could find another way -self creativity- to construct the set of questioning for the IEM Validation *-APPENDIX 1 describes each Cognitive-*.

Next, we present the main findings regarding the use of Intuition in management processes -which are concerned with the structural development of this research proposal towards the IEM, Figure a). *These initial quotations presented below are a starting "to get the things understood" and advancing towards the Intuition Ecosystem Model Validation Process.*

- *According to Guevara [08], "...Men believe that the difficulties originate from one outside, when, in fact, these difficulties are not more than constructions of one own thought, i.e., the man develops an intuitive understanding of the nature of the conflicting forces and the means to anticipate and administer them. Intuition permits clear recognition of these contradictory forces, allowing the release of interactions and renewable energy structures and promoting the fluidity of thought and cooperative work. Intuition and the imagination and the dream play a critical role in rationality, and despite not being able to prove them employing mathematics, Intuition is the driving force for a reason…".*
- *According to Crossan, Lane & White [09], "... Intuition may happen within a group or organizational context, but recognizing a pattern or possibility comes from within an individual. Organizations do not intuit: Intuition is a human attribute that organizations do not possess. Intuition involves some sort of pattern recognition. It is the beginning of new learning that may guide the individual's actions.*
- *According to Alex & David Bennet [10], "...The unconscious works with a processing capability many times greater than that at the conscious level. So, as the world grows more complex, decision-makers will depend more and more on their intuitive, tacit Knowledge. Developing intuitive skills requires making sure that your experiences are meaningful. Understanding the outcomes of actions and why something did or did not happen helps develop patterns in the unconscious (Intuition). Intuitive, tacit Knowledge can be nurtured and developed through exposure, learning, and practice. The relation between tacit & explicit Knowledge and consciousness recognizes that Intuition is typically understood as the ability to access our unconscious mind and thereby effectively use its enormous storeroom of observations, experiences, and Information. Intuition is one of the four ways tacit Knowledge expresses...."*

Finally, regarding our research methodology -section 1.3, Research Methodology- we also surveyed several issues such as human being cognition, intuition processes, pattern recognition, cognitive model, decision making & wisdom quality indicator. The following papers presented below offer a plus guideline for the IEM Validation development:

- *According to* Roger Dolce [11]: *"...Intuition in decision-making is defined as a "non-sequential information-processing mode. It is distinct from the insight and can be contrasted with the deliberative decision-making style. Intuition can influence judgment through either emotion or cognition. In general, individuals use Intuition and more deliberative decision-making styles…"*
- *According to Singhal, Himani & Singh [12]*: "*…Regarding the role of Intuition in Strategic Decision Making, Intuition is a way of knowing without conscious Reasoning. Top-level executives rely on their Intuition to solve complex problems where rationality cannot Help. Intuition involves learned patterns of Information, appears to be a positive force in decision making, and is of positive use in business for decisions involving problem identification, recognizing patterns, and forming strategies in tune with the evolving environment…".*
- *According to Wilder RL [13]: "... Intuition provides a conceptual foundation that suggests the directions which new Research should take by providing the "educated true or false guess. In either case, progress cannot be made without it, and even a wrong guess may lead to progress..."*
- *According to Locke & Chou [14], "...If you only have a small window of time to rely on your Intuition, intuition decision-making can be helpful because it is faster than a detailed analysis. This is especially true when there is very little Information to decide. When Information and time are scarce, Intuition can often be as effective as a rational approach. Intuition is essentially a feeling, and we do not know the source. Before you decide to trust your gut, ask yourself: Am I an expert? Is this an unstructured problem -Cause and Effect- ? Moreover, how much time do I have to choose?..."*
- *According to Lufityanto, Donkin, and Joel [15]:"...Intuition does exist. We can use unconscious Information in our body or brain to help guide us through life, enable better & faster decisions, and be more confident in our choices. Intuition may be improved over time: Intuition can be enhanced with practice and could be applied in the workplace, taking the place of existing questionnaires, which only test people's opinions about their feelings of Intuition. We do have Intuition, and we can measure it scientifically…".*
- *According to Neuroscience News [16]: "...Intuition or gut feelings are also the result of much processing that happens in the brain. Research suggests that the brain is a large predictive machine that constantly compares incoming sensory Information and current experiences against stored Knowledge and memories of previous experiences and predicts what will come next. Clark [17] describes this brain-behavior as the "predictive processing framework, i.e., the brain supports perception and Action by constantly attempting to match incoming sensory inputs with top-down expectations or predictions. Intuitions occur when your brain has made a significant match or mismatch (between the cognitive model and current experience…".*

The above references [11, 17] present an additional guideline about cognition, Intuition, pattern recognition, and cognitive model. References [04] and [06, 10] present the issue regarding intuition use in managerial processes. From these two initial complementary sets of papers, we are sure much more can be discovered, formalized, and improved towards constructing the questioning set for the validation model of our Intuition Ecosystem Model -**IEM**-. *However, again, this is an opinion paper, and, for sure, a Researcher may find a better way to get to the best set of questioning for the IEM Validation Process -Figure a).*

Another branch in constructing the set of questioning is to correlate Guevara's Model [08] with our IEM, "Figure a)." By the same token, pattern recognition and organizational context -as proposed by Crossan, Lane & White [08], are discussed by Balloni and Feldman [01] with a different approach. Analogous to tacit & explicit Knowledge -from Alex & David Bennet [10], both -and much more-have received a different conception by Balloni & Feldman [01]. Finally - last but not least- to correlate the cause and effect discussion presented by Balloni & Feldman [01], in contrast to Locke & Chou [14]. The same Reasoning and correlation between reference [01] and references [02] and [11, 17]. All these correlations should guide us towards the construction of the set of questionings aiming at the validation model of our Intuition Ecosystem Model -**IEM**-

When working on the screening process of Intuition reference searchings, other's correlations towards the set of questioning for IEM validation may come out, such as the human being cognition process and correlation with imagination, aesthetic, pattern recognition, and cognitive model.

So, for successfully to find the best set of questionings for the D, I, K, W, we must advance towards the Intuition studies by carrying out a selection of papers and making the proper correlations & creativity aiming to advance towards the creation of the best set of questioning for the IEM validation. *This opinion paper aims to rescue intuition decision-making by including it in our day-to-day life decision-making processes.*

By last and about the importance of this Research, we have: -"*the most efficient way to improve the decision-making process is improving the Intuition. The people who come with a great repertoire of simple scenarios of Management or games and their respective analyzes have more accurate Intuition and do not forget easily of important aspects that should be considered in the decision about a problem*", by Reinhard Selten [04]. So, this research proposal is an answer to Selten [04]: the development of an "*An Intuition Ecosystem Model Validation for Enhancing General Decision Making -IEM Validation-"*

The Intuition Ecosystem Model and its Validation Process

The bottleneck of this work is the Intuition Ecosystem Model Validation -IEM-. This Validation process must be guaranteed by the best set of questions. Through out this paper has proposed a roadmap for creating a Validation Process for our Intuition Ecosystem Model -Figure a)-, which should enhance general intuitive decision-making. Therefore, the creation of the new Knowledge -the creation of the set of questions for the IEM validation process- should come from four different approaches -as discussed previously-:

1. The references searching and correlation analyses and,

2. Self-creativity to construct the validation process based on your own life and CV experiences, PLUS Ideas exchanges and participation from other fellows, researchers, and mentors.

3. Understand the critical aspects of your market business or research -its local or global trends or patterns...

4. Understand the meaning of each Cognitive Unite DIKW -see Appendix I-.

A plus expectation for any Researchers interested in this opinion paper is their own life and CV experiences. Each Researcher has a unique ecosystem from which it is possible to create and advance toward the Validation Model for IME. The Researcher's background offers him a safe and unique position to construct the set of questioning by applying his interdisciplinary life experience towards this challenging scientific development and getting the instrument for Validation of the IEM -Figure a)- aiming at enhanced general intuitive decision making.

In short, based on the Researcher's previous experiences and accountability way of life, this researcher may be able to improve his determination, focus, creativity, balance, and innovation resulting in the development of the Intuition Ecosystem Validation for Enhancing General Decision Making. *See the definitions of each cognitive unit in APPENDIX 1.*

Effectiveness of the proposal for developing the validation tools -IEM Validation- and Risk Management

In this opinion paper, we have raised the issue of TESTER. Testers are the ones who will implement the VALIDATION MODEL -to implement a set of questionings- Specifically, in section 1.2/e.1, we have mentioned about 50 TESTER.

Consideration to be taken into account by the researcher working towards the finding of the Validation Model: *Which percentage of Testers applied the Validation Model? For example, in a sample of 50 testers, 75% YES, 15% NO, and 10% INCONCLUSIVE and WHY.*

Risk Management of the IEM Validation Instrument.
Concerns for the **contingency plan for the IEM VALIDATION** are listed below in Table 1:

Table 1. Risk contingency plan for the IEM VALIDATION (Risks when constructing the IEM VALIDATION MODEL -the set of questions)

Identified Risk	Risk Potential	Adopted solution
Failing in developing the Intuition Ecosystem Model Validation. -	Low	Verify that you have adopted all suggestions presented in this opinion paper were considered in full.
Failing in Finding the 50 testers available for validating the Intuition Model.	Medium / Low	Look for new testers; replace the category of a tester. At first, the Tester should belong to the ecosystem of the Researcher. If needed, we must look for new testers out of this ecosystem. The maximum Tester should comply with 50.
Failing to have the Intuition Ecosystem Model & Validation Process published in an Indexed Open Access Journal or Congress Proceedings.	Low	Look for other thematically similar publications, Journals, or Congress Proceeding

Discussion & Conclusion

Structural Development: Impacts and Perspectives

In this opinion paper, we have proposed a road map to find the IEM Validation -a set of questioning for each Cognitive Unit (D, I, K, W). APPENDIX 1 describes these cognitive units.

a. Our premise is that the Intuition Ecosystem Model Validation Process will be the most powerful instrument to enhance general intuition decision-making once developed. It should fill an existing gap in general decision-making theory and interdisciplinary Research and open new perspectives to Research concerning general IEM applications & implications
b. With the IEM Validation Process at hand, it will be possible to make a general decision making in any field of life and its applications to economic and social issues. The Management of the economy reflects a social value-adding -the multi/interdisciplinary aspects of the IME application-. Therefore, the Development of an IEM Validation Process is the first endeavor and innovation toward general Management. As an interdisciplinary and multidisciplinary approach, the IEM Validation Process must impact several economic societies & research branches. Its implementation is for any decision-making, such as research & innovation management, education, strategic & systemic innovation. *The author of this opinion paper also believes the IEM Validation may be an instrument for societal transformation and sustainable development towards the political processes, mis- and disinformation (fake news has been the tone lately)*, democracy, and equality.
c. Finally, the IEM Validation must open the way for other research groups, from disciplines such as SW development, Education, artificial intelligence, or economy, by developing new perspectives on management decision-making approaches and triggering innovative collaboration opportunities. *Therefore, we call interested researchers to work on this challenge of finding the Validation Process for the Model of Figure a)* and help construct a better and more prosperous and adding value society.

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Appendix I. For Figure a) of this opinion paper, and, according to Balloni & Feldman [01 and 18], we present the following *general definitions for the* ***Corollary Proven Cognitive Units*** *-D, I, Kie, Wie-:*

D -DATA-: "is a measuring Cognitive Unite -CU-that describes the Information of raw facts. It is not a function of context, and it is not a pragmatic unit since it involves only measurements. When moving from Data to Information involves understanding context. Data may trigger the Wisdom -"...vision & foresight..."-, as discussed in section 2.1, from reference [01]. On the other side, Wisdom may not trigger or become Data: Wisdom is a pragmatic cognitive and context-dependent unit that involves ultimate Action towards decision-making. Data is not a function of context, and it is not a pragmatic unit since it means only measurements of raw facts. Therefore, Data may trigger Wisdom and never vice versa."

I -INFORMATION-: "It is a comparative and pragmatic CU, which is meaningful and useful to a human being -HB- in a specific context. Moving from Information to Knowledge involves understanding patterns. Therefore, for the same reasons presented for Data, Information may also trigger Wisdom in a specific context - see section 2.1, item C, reference [01]. However, Information never becomes Data. Why? Because Data is not a function of context, and Information is context-dependent. Information is irreducible to Data."

K -KNOWLEDGE-: " is a reasoning and pragmatic CU created by applying human experience to available Information -the external or internal Human Being -HB- context applied to the available Information. As an internal HB process, it is a guide for Action, i.e., when moving from Knowledge to Wisdom involves understanding Concept - C- and Principles. The Concept represents the Reasoning encompassing the Knowldege -not by taking into account what we know and instead by how we use this Knowledge-. Reasoning requires considering the coupling of Knowldege with a Mindset: "the ideas and attitudes with which a person envisions to deal with a situation." This Mindset comes from the philosophical Wisdom -W- definition proposed by the authors [01]: Wisdom –W- is the capacity to put into Action an acquired knowledge. This Action implies correct judgment and requires the understanding of the coupling of Knowledge with the following Principles: Competence, Prudence, and Imagination -CpPI-before an ultimate action towards decision-making. Therefore, the Mindset has embedded these Principles: CpPI. The coupling of Knowledge with the Mindset -CpPI - must be considered before presenting a set -when possible a set- of possible solutions regarding decision-making towards Wisdom.
Knowledge also never becomes Data. Why? Because Data is not a function of context, and Knowledge is context-dependent."

"**Ki,e -Knowledge: Implicit, Explicit-:** besides the definitions presented in section 2.1 [01], we should also emphasize when **Ke** is delivered (told or written) it becomes an **I**: when you tell someone something, you give them **I** which may -or not- create new **Ki,e**."

"**Implicit K**nowledge -**Ki**: **practical, action-oriented K, or 'know-how'** based on practice, acquired by personal experience, seldom expressed openly, often resembles Intuition since it is part of the human mind." [01].

"**E**xplicit **K**nowledge -**Ke-**: **academic K, or 'know-what'** that is described in a formal language, print, or electronic media, often based on established work processes, use people-to-documents approach and, as such K^e is designed for sharing." [01].

W -WISDOM-: "is a pragmatic cognitive and context-dependent unit not found in Knowledge. In spite of Knowledge delivering all you need for final Action, it is in the Wisdom where the ultimate Action effectively occurs: the capacity to put in Action an acquired Knowledge (see Concept -C- definition). As already mentioned, this Action implies correct judgment and understanding of the coupling of Knowledge with the principles CpPI -the mind-set-before an ultimate action toward decision-making.."

Wi,e -Wisdom: Implicit, Explicit-: finally, by considering the definitions for DIKW presented in section 2.2, [01], we may affirm **that Wisdom may become Knowledge and** may become **Information. Indeed,** we must emphasize what today is **Wisdom** may, **through the time**, become Information or Knowledge-. See items A), B) and C), section 2.1, [01].

"**We** is manifested through the wisest Action -you made the decision to invest money in a specific issue-

Or

Wi it is part of the human mind since it could only be visualized as another - and wise- possible decision to invest the same money in a different issue as that from **We**." [01].

We have an interdependent and intertwined character among D,I, Ki,e,Wi,e – [01].

Electrical Conductivity and Activation Energy of Heteroepitaxial Diamond

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Abstract: Diamond is an attractive material for high power electronic devices because of its unique properties such as wide band-gap and high thermal conductivity. Heteroepitaxial growth of diamond offers the possibility of making devices over a large surface area. In order to address its suitability for electronic application and characterize electrical properties of heteroepitaxial diamond, a device is fabricated from a heteroepitaxial diamond film. Here the temperature-dependent DC electrical conductivity of heteroepitaxial diamond is reported. Owing to its extremely low electrical conductivity at room temperature, measurements of current-voltage characteristics were made between 300 ºC and 550 ºC. The technique used to measure electrical conductivity of heteroepitaxial diamond at high temperature required precise measurement circuits enabling thermal control with sufficient stability for electrical measurements of very small currents ($<10^{-13}$ A). Ohmic contacts with diamond and procedures for preparing diamond surface are reported. In this study, an activation energy of 1.40 eV is found for the diamond device. This activation energy is close to that of natural diamond in medium range temperatures, between 400 to 550 ºC. The activation energy of the heteroepitaxial diamond appears to be reduced to ~1.3 eV at lower temperatures (<350 ºC), which matches the previously studied homoepitaxial diamond, suggesting the presence of electronic states comparable with homoepitaxial diamond.

Keywords: Diamond, Electrical conductivity, Activation energy, Semiconductors

Introduction

Diamond is a wide band-gap 5.5 eV semiconductor, with attractive electronic properties for electronic applications. These include low electrical conductivity of 10^{-16} $(\Omega\text{-cm})^{-1}$ at room temperature [1], a large dielectric breakdown field of 10^7 V/cm [2], and carrier mobilities as high as 4500 cm^2/V-s and 3800 cm^2/V-s for electrons and holes, respectively [3]. The high carrier mobilities have been attributed to the high purity of synthetic diamond produced by a homoepitaxial chemical vapor deposition (CVD) process [3].

A synthetic diamond could facilitate a controlled condition to obtain a diamond with these desired properties. Growth of homoepitaxial diamond for large size electronic application requires a diamond substrate with high quality and purity. An alternative to homoepitaxial growth is high quality heteroepitaxial growth of diamond on iridium [4-8], which could lead to wafer-scale diamond substrates with smooth surfaces suitable for electronic

Behravan, M. (2022). Electrical Conductivity and Activation Energy of Heteroepitaxial Diamond. In C.L. Zeman & H. Akcay (Eds.), *Proceedings of ILSET 2022-- International Conference on Life Sciences, Engineering and Technology* (pp. 43-49), Los Angeles, USA. ISTES Organization.

applications. As such, it is of great interest to examine electrical properties of diamond grown by alternative methods such the heteroepitaxial diamond sample in this study.

Because of low conductivity of diamond, the electrical measurement must be made at elevated temperatures [9]. In an early investigation, measurements of the DC conductivity of natural Type IIa diamond, i.e., diamond with substitutional nitrogen impurities at the ppm level, were made above room temperature [1, 10-12]. This paper reports on the electrical conductivity of heteroepitaxial diamond at high temperatures measured by a unique technique and the corresponding activation energy. Ohmic contacts with diamond and procedures for preparing diamond surface are addressed.

Method

Diamond Sample Preparation

A heteroepitaxial diamond grown by bias enhanced microwave plasma Chemical Vapor Deposition (CVD) on 250 nm Ir /(001) SrTiO3 surface [8] was used for measurements. The diamond sample- with 3.5 mm diameter and an average thickness of 20 μm- is shown in Figure 1a. Initially, four electrodes were deposited on the diamond sample for surface leakage tests (Figure 1a). The dark-spotted areas on the diamond sample are inclusions with macroscopic defects [9]. Spiral-type defects, with average sizes of about 10-20 microns, are detected in the optical microscope images, as shown in Figure 1b.

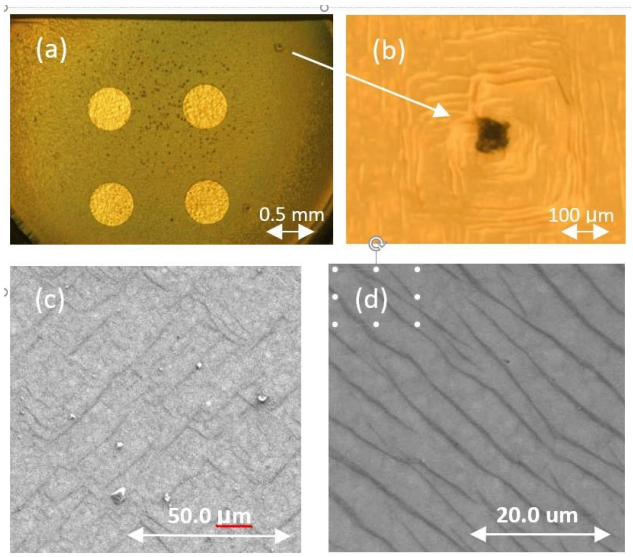

Figure 1. Optical microscope image of (a) the free-standing sample at 5X with average diameter of 3.5 mm and an array of four 0.5 mm diameter Ti/Au electrodes, and (b) spiral type defect with average size of ~ 50-100 μm at 20X; SEM images of diamond surface (c) near the center showing inclusions and screw dislocations, and (d) away from the center with fewer defects showing macrosteps parallel to [110] with an average size of 5 μm.

Further examination of the sample by SEM images of the surface revealed inclusions and screw dislocations,

shown in Figure 1c, and macrosteps textures parallel to [110] with fewer defects, shown in Figure 1d. The size of the inclusions ranges from 1 μm to 10 μm and the average size of the steps is 5 μm. An average thickness of 20±1 μm was found for the diamond piece by SEM cross-sectional examination of the sample.

Electrical Measurements

Due to the low conductivity of diamond, current-voltage measurements require considerable care to avoid extraneous current leakage resulting from surface conduction [9] that may originate from contaminants, non-diamond carbon, and hydrogen adsorbed on the diamond film surface [12].

One of the experimental problems in determining DC conductivity of synthetic diamond is the high surface conductivity of the as-grown film. The surface conductivity of diamond following hydrogen exposure during growth is high, with transport through surface states governed by a very low activation energy ~ 9-23 meV [13]. To remove the surface leakage caused by either hydrogen termination or any surface contamination, the sample was exposed to an oxygen plasma for 30 seconds after a series of surface treatments.

To examine the surface conductivity, an array of Ti/Au electrodes is fabricated on the top surface of diamond sample, as shown in Figure 1a. For the top electrodes, a thermal evaporator with base pressure 10^{-7} Torr was used for consecutive Ti (100 nm) and Au (250 nm) depositions. Prior to deposition, the diamond surface was cleaned with a combination of acids to remove surface residues [9]. Following the metal deposition, the sample was annealed at 650 ºC for 20 minutes at 2×10^{-8} Torr to form the Ohmic contacts between Ti and diamond surface by carbide formation [14]. Finally, the sample was treated with an oxygen plasma for 30 seconds to remove the surface leakage caused by surface alteration, hydrogen termination or any surface contamination, and minimize current leakage.

Since diamond was grown on Ir epitaxially, and the Ir-diamond contact was found to be Ohmic [12], the Ir covering the entire diamond back surface was left in place to serve as the back contact. In order to make contact to iridium, the sample was placed on a silicon substrate on which an Au pad was deposited.

For testing surface leakage, I-V measurements at room temperature were taken in an electrically shielded probe station before and after oxygen plasma exposure. With bias in the range of ±3 V, the current is in the range of μA prior to oxygen treatment, and it was reduced significantly to the noise level of the electrometer ~0.1 pA following the oxygen plasma treatment. This is interpreted as the elimination of surface conduction, and evidence that the conductance could not be measured at room temperature.

Since the bulk conductivity of diamond is low and the diamond sample is thin ~ 20 um, the electrical contacts on the opposing surfaces maximized the current flow in the perpendicular direction. A piece of the sample with significantly fewer defects was cleaved off and was used for the present electrical measurements. Another piece of the sample was tested as a particle detector device with sub-nanosecond response times at high beam

intensities [15] after completing high-temperature electrical measurements of heteroepitaxial diamond [9].

For the electrical measurements up to 600 ºC, a setup for holding the sample, electrical contacts and wires, heating system, and thermocouple was designed and built (inset in Figure 2). The diamond sample was placed on an Au pad that was deposited on a sapphire substrate. A thermocouple was placed in the vicinity of the sample on the sapphire. Three mini-heaters in series with a power of 4.3 W served to heat the setup to 600 ºC. The sample setup was held together mechanically using stainless steel clips. To prevent noise interference with the measurements and to prevent any leakage, the sample setup was suspended in a grounded aluminum box (Faraday Cage) equipped with coaxial fittings and wiring [9].

Since diamond reacts with air above 500 ºC, all measurements were performed in a vacuum of 2 x 10^{-7} Torr. A variable DC voltage source in conjunction with an electrometer (Keithley-6514) with 0.1 fA resolution and 1% accuracy were used.

Results and Discussion

I-V characteristics for bottom (Ir) and top (Ti/Au) contacts were Ohmic between 300 and 545 ºC for voltages between -0.5 and 0.5 volt. Figure 2 shows I-V plot for 545 ºC. At higher bias voltages, deviation from the linear behavior was observed – indicating a non-ohmic contact.

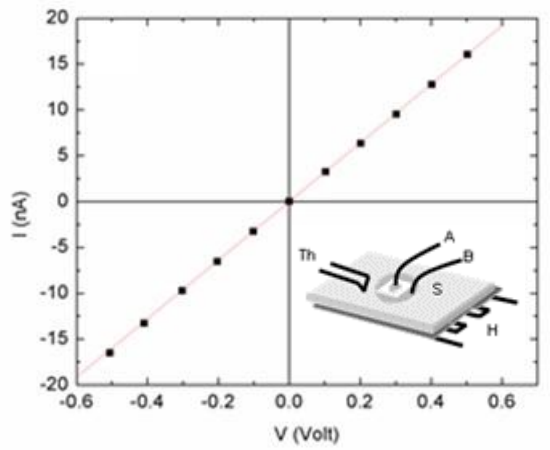

Figure 2. I-V characteristics of heteroepitaxial diamond with Ohmic Ti/Au and Ir contacts at 545 ºC, and Inset: sample holder schematic; Sapphire 10 mm square; white square, the diamond sample; A, Au-wire contacting top electrode; B, Au-wire contacting Au-pad; Th, Thermocouple; S, Sapphire; H, Heater.

The conductance was obtained from a linear least-square fit to the I-V data for different temperatures. It was found that the DC conductivity of heteroepitaxial diamond is strongly temperature dependent, increasing by four orders of magnitude, from 6.80×10^{-12} $(\Omega.cm)^{-1}$ at 300 ºC to 3.20×10^{-8} $(\Omega.cm)^{-1}$ at 545 ºC, as shown in Figure 3.

To find the activation energy E, a least-squares regression line was applied to the conductivity data in Figure 3 - the dashed line. An activation energy $E = 1.40 \pm 0.03$ eV is found using the equation $\ln\sigma = \ln C - E/k_B T$, where E/k_B and ln C are the fit parameters, and E and k_B are the thermal activation energy and the Boltzmann constant, respectively.

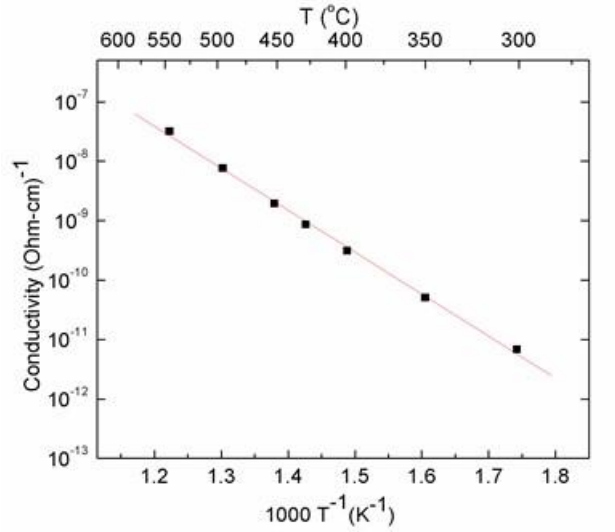

Figure 3. Conductivity of heteroepitaxial diamond vs. T^{-1}. An activation energy of 1.40 ± 0.03 eV is found for heteroepitaxial diamond from the linear fit (solid line).

The temperature-dependent DC conductivity of the heteroepitaxial diamond is compared with prior measurements reported on natural diamonds: Type IIa crystals, Type Ib crystal (Figure 4), and homoepitaxial diamond. For heteroepitaxial diamond, we found an activation energy of 1.40 ± 0.03 eV between 300 and 550 ºC. Vandersande and Zoltan (VZ) quoted $E = 1.4$ eV for Type IIa diamond between 300 and 1200 ºC [1]. However, the activation energy appears to be ~1.8 eV at higher temperature between 600 and 1200 ºC and ~1.4 eV between 400 to 550 ºC for VZ Type IIa diamond. Borst and Weis (BW) quoted an activation energy of 1.7 eV for Type IIa and Ib, and 1.3 eV for homoepitaxial diamond between 180 to 350 ºC [10].

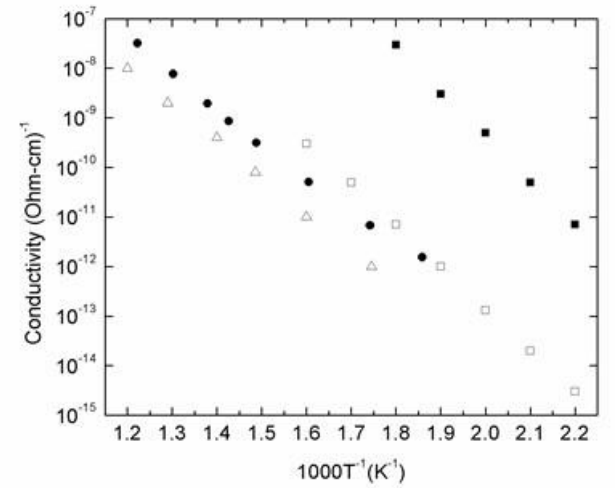

Figure 4. Comparison of temperature-dependent conductivities of heteroepitaxial ●, natural Type IIa Δ [1], Type IIa □ and Type Ib diamond ■ [10]. VZ and BW plots are reconstructed from VZ report Figure 4 [1] and BW report Figure 1 [10], respectively

The activation energy of 1.7 eV has been related to the substitutional nitrogen donor [10]. The observed conductivity difference of four orders of magnitude between the BW Type Ib diamond and the BW Type IIa diamond may be explained by the greater number of nitrogen donor electrons in Type Ib.

The activation energy of ~1.3-1.4 eV could be related to defects e.g., hydrogen vacancy center defects. Natural diamond and CVD diamond (polycrystalline [16] and heteroepitaxial diamond [17]) may contain concentrations of hydrogen. When a carbon vacancy occurs, the hydrogen atom bonds to a single carbon, leaving two bonded carbons and a carbon dangling bond. This defect does not show signs of annealing out at temperatures up to 1500 ºC [16] and could play a role in the conduction mechanism.

Conclusion

This study presented the measurement method for the electrical conductivity of heteroepitaxial diamond and showed Ohmic behavior for Ir and Ti/Au contacts between 300 and 545 ºC for -0.5 to 0.5 volt. This investigation has demonstrated that the electrical conductivity of CVD-grown heteroepitaxial diamond is thermally activated, with an activation energy of 1.4 eV above 350 ºC, and appears to decline to a smaller activation energy ~1.3 eV at temperatures below 350 ºC.

A summary of this study and each of the studies included by reference reveals a consistent pattern: (1) natural diamonds –Types IIa and Ib- provide for electron activation energies determined by nitrogen substitution (E = ~1.7-1.8 eV), and (2) synthetic diamonds - both homoepitaxial and heteroepitaxial - provide for electron activation energies ~1.3-1.4 eV attributed to defect states that lie between the conduction band and the nitrogen substitution band.

The similarity between heteroepitaxial and homoepitaxial diamond activation energies is due to comparable electronic states in defect bands associated with both heteroepitaxially and homoepitaxially grown diamond - observed spiral defects and inclusions in the heteroepitaxial sample related to states introduced by carbon vacancies and corresponding hydrogen vacancy centers.

This result indicates the presence of heteroepitaxial diamond defect states lodged between the conduction band and the nitrogen substitution levels that are evident in natural diamond Type IIa.

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Gas Chromatography-Mass Spectrometry and High Performance Liquid Chromatography Analyses of *Persea Americana* (*Lauraceae*) Seeds Extract

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Abstract: Plants have been found useful in the treatment of various diseases affecting man. Several pharmacological activities of aqueous seed extract of *Persea Americana* (Avocado) (ASEPA) has been reported. Pharmacological investigations had attributed strong anti-ulcer activity to ASEPA. High performance liquid chromatography (HPLC) for its major compounds and the GC-MS analysis (gas chromatography-mass spectrometry) for structure elucidation of compounds in the seed extract is yet to be carried out. Knowledge of the major compounds might guide scientists to a novel anti-ulcer drug. The aim of the study was to carry out high performance liquid chromatography (HPLC) and GC-MS study on the aqueous seed extract of *P. Americana* (ASEPA) and fractions. Dried and pulverized seeds of *P. americana* was soaked in distilled water (25 ^{o}C) for 24 hours, filtered and freeze-dried. The extract was fractionated with n-hexane and residue was the water fraction**.** Analytical high performance liquid chromatography (HPLC) was used for identification of the major compounds. HPLC was able to identify polar compound. sample from VLC analysis was used for GC-MS analysis using Shimdzu (GCMS-QP2010plus) system in order to identify non-polar compounds in the extract and fractions. HPLC analysis revealed major polar compound as Abscisic acid while GC-MS analysis elucidated structures of 24 monosaturated fatty acids. *P. americana* seeds extract and fraction contain mainly Abscisic acid and long-chain monosaturated fatty acids

Keywords: HPLC, Gas column-mass spectrometry, Abscisic acid, Monosaturated fatty acids

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Introduction

Medicinal plants have continued to attract attention in the global search for effective methods of using plants' parts for the treatment of many diseases affecting humans (Alexandra *et al.*, 2018). Many important drugs used in medicine today are directly or indirectly derived from plants due to their bioactive constituents such as; alkaloids, steroids and tannins (Thomford *et al*., 2018)**.** According to Umeh *et al*., (2020**)** aqueous seed extract and fractions of *Persea americana* and its fractions (n-hexane and water) exhibited dose-dependent significant ($p<0.05$) reduction in ethanol, NSAID and stress induced ulcer indices at 250 and 500 mg/kgbw; revealing the ulcer protective effect of this extract and fractions. These extract and fractions also conferred a dose-dependent healing and reduction in ulcer index in all the three ulcer models (ethanol, asprin and stress). The n-hexane fraction at 500 mg/kg exhibited stronger ulcer-healing and ulcer-protective effects than the standard, Cimetidine 150 mg/kg (Umeh *et al.*, 2020, Okoye *et al.*, 2021). There is therefore the need to study the compounds in this n-hexane fraction of *Persea americana* which might be responsible for its ulcer healing and ulcer-protective effects. Bearing in mind herbal medicine is becoming a viable alternative treatment over the commercially available synthetic drugs for peptic ulcer (PU) management and treatment (Keshavarzi *et al.*, 2014, Hashemi *et al.*, 2015).

Method

Collection and Identification of Plant Material

The fruits of *P. americana* were purchased from Oye Nimo, Nimo, Njikoka local government area Anambra State, Nigeria in the fruiting season of April 2016. It was identified and authenticated by Dr J. E. Amadi, a Taxonomist at the Department of Botany, Nnamdi Azikiwe University Awka, Nigeria and a herbarium specimen number NAUH.13, was assigned to it and kept in the hebarium.

Extraction of *Persea Americana* Seeds

The seeds of *P. americana* were removed from the fruits and chopped into small pieces, shade-dried for 5 days and grounded into fine powder with Binatone blender (Model BLG-401). The powder (I kg) was soaked in 2 litres of distilled water for 24 h. This was first filtered by passing it through a cotton plug and further filtered with filter paper (Whatman filter paper, No 1). The aqueous seed extract of *Persea americana* (ASEPA) was freeze-dried to a constant weight and stored at 4 °C in a amber-colored bottle until required for experiments.

Fractionation

To 100 g of freeze-dried ASEPA in a mortar, 100 ml of distilled water was added and mixed thoroughly. This was then poured into a separating funnel and another 100 ml of distilled water was added and mixed by shaking vigorously. Then, 250 ml of n-hexane was added and used to wash the extract by shaking vigorously. After 30

minutes the n-hexane layer was collected into a beaker. Subsequently, more 250 ml volumes of n-hexane were added and collected as before until the n-hexane layer became clear. The residue which was the water fraction was then removed from the separating funnel. The two fractions, water fraction (WF), and n-hexane fraction (NF) were dried to a constant weight using rotary evaporator at 40^{o}C.

HPLC Analysis

Analytical high performance liquid chromatography (HPLC) was used for identification of the major compounds. Each of the dried extracts (ASEPA, NF, and WF) was reconstituted with 2 ml of HPLC grade methanol. The dissolved samples (100 µL) were each transferred into HPLC vials containing 500 µL of HPLC grade methanol. HPLC analysis was carried out on the samples with a Dionex P580 HPLC system coupled to a photodiode array detector (UVD340S, DionexSoftron GmbH, Germering, Germany). Detection was at 235, 254, 280 and 340 nm. The separation column (125 cm × 4 mm; length × internal diameter) was prefilled with Eurospher-10 C18 (Knauer, Germany) and a linear gradient of nanopure water (adjusted to pH 2 by addition of formic acid) and methanol was used as eluent

Vaccum liquid chromatography (VLC) of NF

The VLC analysis of n-hexane fraction was carried out according to methods described by Cruz et al., (2016). The n-hexane fraction (6 g) was mixed with 300 g silica gell (mesh 200-400), air-dried and eluted with 500 ml of various ratio of n-hexane: ethyl acetate mixture (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10). These resulted into 11 fractions. Thin layer chromatography (TLC) of the eleven fractions was carried out to confirm presence of compounds.

Structural elucidation of compounds using GC-MS

Out of the eleven VLC eluents, only one yielded an oily compound after drying. Others were either too negligible or did not yield any compound at al. Therefore, the oily sample was subjected to gas column-mass spectrometry (GC-MS) analysis using Shimdzu (GCMS-QP2010plus) system Tokyo, Japan equipped with a AOC-20i auto-sampler according to the method described by Pripdeevech *et al* (2010). The columns used were an Rxi-sms capillary column (30m x 0.25 mm x 0.25 µm) (Belonite, PA, USA). The stationary phase was 5 % two phenyl, 95 % two methyl polysiloxanewhile and Helium was the carrier gas (0.7 ml/min). The injector temperature was 250 °C and the column temperature was maintained at 40 °C for 5 min and then programmed at 4 °C/min to 250 °C.

The spectrometers were operated in electron ionization (EI) mode at 70 eV ionization energy; the scan range was 35–400 amu. The detector was set as fixed voltage at 1200V and the scan rate was 0.5 s per scan. The ionization source temperature was 250 °C. The identification of the major compounds was performed by comparing their mass spectra with the Wiley Registery of Mass spectra 8th edition library available in the

instrument and confirmed by comparing with standards from national institute of standards and technology, mass spectra libraries (NIST).

Results

Absciscic acid was found to be the major compound present in the aqueous crude extract and fractions of *P. americana* seeds as can be seen in figures 1B, 2B and 3B. The GC-MS Spectra of the phytochemical constituents from n-hexane fraction revealed 24 compounds. Decane was the most abundant (9.99% of total oil composition) with retention time of 6.84 minutes while the least was 2,4 dimethyl heptane (1.57 % of total oil composition) with retention time of 4.6 minutes. All the compounds were saturated fatty acids (alkanes) (Figure 4, Table 1).

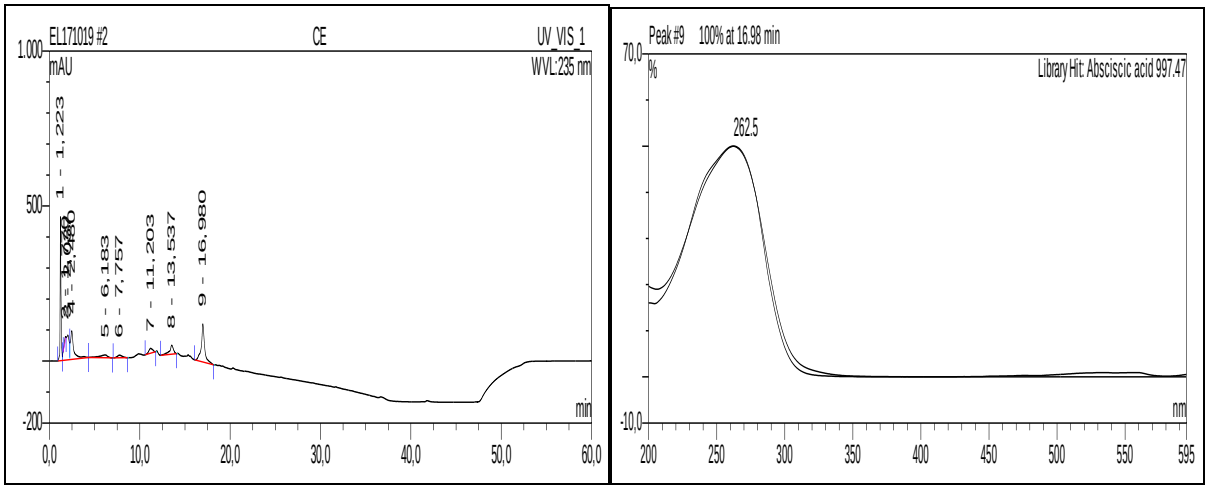

Figure 1A & 1B. HPLC Chromatogram of ASEPA and UV Spectrum at peak 9 respectively; suggesting Absciscic acid at 235 wavelength. Key: CE=Crude extract (ASEPA**).**

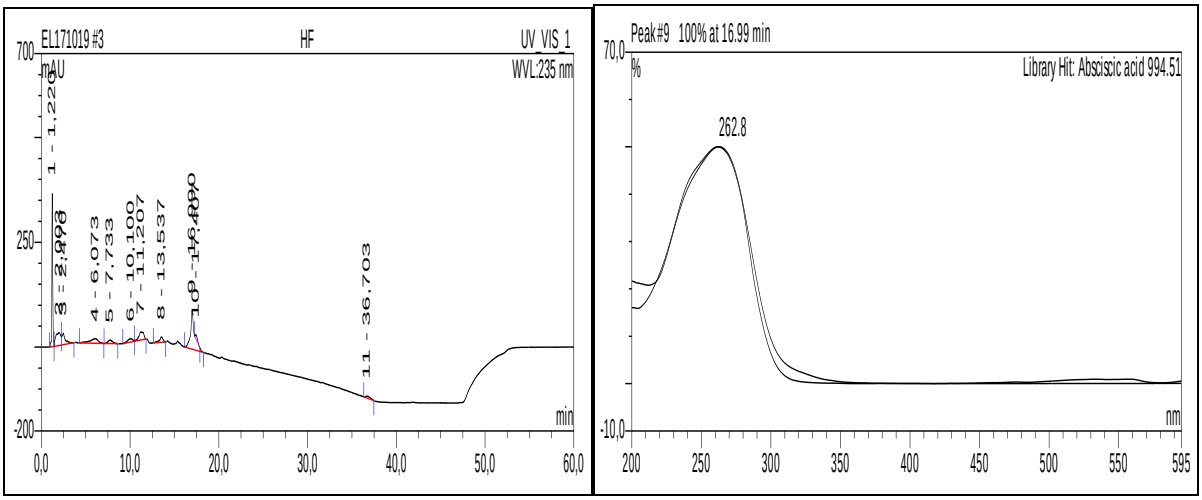

Figure 2A & 2B. HPLC Chromatogram and UV Spectrum of Peak 9 respectively; suggesting Absciscic acid at 235 wavelength. Key: NF= n-hexane fraction (HF)

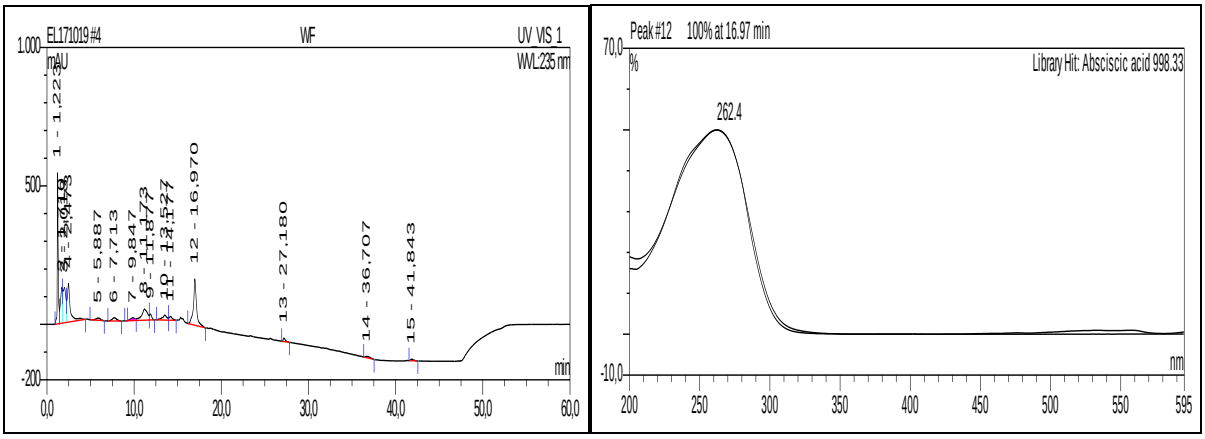

Figure 3A & 3B. HPLC Chromatogram and UV Spectrum of Peak 12 respectively, suggesting Absciscic acid at 235 wavelength. Key: WF= water fraction

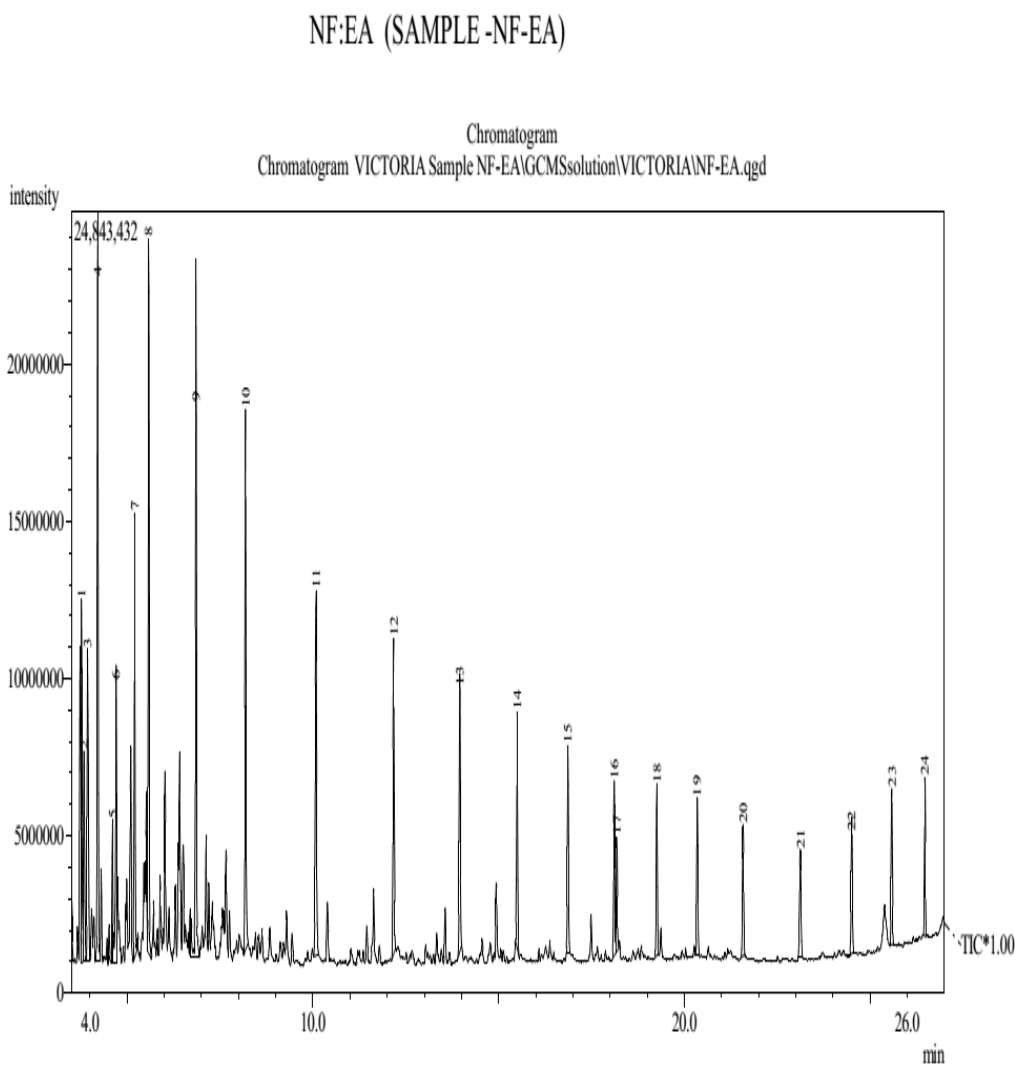

Figure 4. Chromatogram showing 24 major Compounds from GC-MS Analysis of NF

Table 1. The 24 Compounds from GC-MS Analysis of n-hexane Fraction

S/n	Name of compound	Retention time(min)	% Peak area	Molecular weight
1	Methyl benzene	3.76	4.12	92.14
2	3 methyl heptane	3.83	2.77	114.23
3	p-dimethyl cyclohexane	3.93	4.93	112.22
4	Octane	4.2	9.38	114.23
5	2, 6-dimethyl heptanes	4.6	1.57	128.26
6	1 ethyl 3 methyl cyclopentane	4.69	4.03	112.22
7	1,3-cyclopentadine	5.2	5.95	106.17
8	Nonane	5.58	8.38	128.26
9	Decane	6.84	9.99	142.29
10	Decane	8.18	7.39	142.29
11	Decane	10.08	6.42	142.29
12	Tridecane	12.18	4.92	184.37
13	Tridecane	13.96	4.05	184.37
14	Tridecane	15.5	3.35	184.37
15	Hexadecane	16.87	2.93	226.45
16	Hexadecane-	18.10	2.47	226.45
17	2, 6, 11-trimethyl dodecane	18.18	1.67	212.42
18	Hexadecane-	19.26	2.28	226.45
19	Hexadecane	20.34	2.34	226.45
20	Hexadecane	21.58	2.24	226.45
21	Hexadecane	23.13	2.20	226.45
22	Hexadecane	24.5	2.25	226.45
23	2-methyl nonadecane	25.58	2.25	282.56
24	2, 4-dimethyl eicosane	26.478	2.13	310.61

Discussion

The HPLC identifies polar compounds which can be detected by UV light. The results of HPLC study showed the chromatogram of the crude extract (ASEPA) with 9 peaks which represents nine compounds with the most prominent peak being peak number 9 (Figure 1A). For peak 9 the corresponding UV lamda max of 262.5nm at 235 wavelengths suggested Absciscic acid (Figure 1B). The n-hexane fraction (NF) yielded 11 peaks representing eleven compounds. The most prominent peak was peak number 9 (Figure 2A) with the corresponding UV lamda max of 262.8 nm at 235 wave length suggesting Absciscic (Figure 2B). In the same way the water fraction (WF) revealed 15 peaks representing fifteen compounds with the most prominent being peak number 12 (Figure 3A) having the corresponding UV lamda max of 262.4 nm at 235 wave length

suggesting Absciscic acid (Figure 3B). Hence, Absciscic acid was found to be the major compound present in the aqueous crude extract and fractions of *P. americana* seeds. Though the chemical synthesis of Absciscic acid has been developed (Constantino *et al.*, 1986), not much pharmacological studies have been done on Abscisic acid. Bassaganya-Riera *et al* (2010) reported some pharmacological activities of Absciscic acid.

The GC-MS was used to analyze the oil (volatile oil) compound from VLC. The GC-MS analysis of n-hexane fraction revealed 24 monosaturated alkanes (Table 1). Akpabio *et al*., (2011) had reported the presence of monosaturated fatty acids in *P. americana* fruits. Apart from alkanes being precursor for the synthesis of many biologically active compounds, their anti-microbial and anti-oxidant properties have been reported (Jae-Suk Choi *et al.*, 2012, Zaha *et al*., 2016). The ability of fatty acids to significantly inhibit gastro-intestinal motility has also been documented (Zaha *et al*., 2016). It could be possible the ulcer-healing and ulcer-protective activities of n-hexane fraction (Umeh et al., 2020) could be attributed to these compounds in the seeds of P.*americana.*

Conclusion

The major compounds in *Persea americana* seeds are Absciscic acid (polar compound) and monosaturated alkanes (non-polar compounds). The synergesic effects of these compounds may be responsible for pharmacological activities associated with Persea americana seed extract which include ulcer-protective and ulcer-healing properties.

Recommendations

We recommend the testing of chemically synthesized Absciscic acid activity on different ulcer models with the aim of developing a novel anti-ulcer agent.

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Student Perceptions of Low-Code Prototyping: A Case Study

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Abstract: In response to the dire labor market shortage in Information Technology (IT), low-code environments that allow users to quickly develop applications without learning programming are skyrocketing in popularity, since low-code reduces the need for professional software developers. The demand for low-code skills is rising. Based on this growth, universities can provide students with a definite skills advantage as they enter the IT workforce by teaching low-code development. This descriptive case study explores students' perceptions of developing prototypes within a low-code environment in a Systems Analysis and Design (SA&D) course. Students were surveyed to gather their perceptions of prototyping in a low-code environment. The data was analyzed using thematic coding. The results show that while students' perceptions of low-code prototyping were positive, with the majority of students agreeing that low-code was easy-to-use and helpful, there were also drawbacks, such as missing the opportunity to develop coding skills and limitations of the low-code environment. While low-code environments are useful for creating prototypes, faculty should be cautious in replacing traditional coding skills entirely with low-code. The significance of this study is that the results, coupled with the curricular artifacts, provide faculty with the impetus and tools to implement low-code prototyping in their courses.

Keywords: Low-code, Software development, Prototyping, Systems analysis and design, Higher education

Introduction

The number-one risk to companies across the world is a shortage in software development talent, according to Information Technology (IT) research organization the Gartner Group (Pham, 2021). By 2030, the global IT talent shortage is predicted to reach over 85 million (Kosenko, 2020). Over the next ten years, the United States' labor market will add 316,000 software developer jobs while the number of new graduates in Computer Information Systems (CIS) programs is predicted to shrink by 43%, according to the Bureau of Labor Statistics (BLS) (Breaux & Moritz, 2021). This will exacerbate the already dire IT skills gap (Muraski et al., 2021; Ozkaya, 2022).

Low-code development environments have emerged as a potential means to alleviate this shortage. IT professionals utilize low-code environments to build applications more rapidly than with traditional, complex Integrated Development Environments (IDEs) (Fryling, 2019; Sahinaslan et al., 2021).The market for low-code

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development is predicted to grow 23% over the next year, due to the simplicity and speed of developing applications in low-code environments (Costello & Rimhol, 2021). Companies need IT employees who understand low-code environments to address the escalating developer shortage (Cush & Aldana, 2021; Glasscock, 2021). Therefore, higher education can provide students with a distinct skills advantage as they enter the IT workforce by incorporating low-code environments into the curriculum. Over the past two years, universities are beginning to integrate low-code into their courses, particularly for use in prototyping software applications (Lebens & Finnegan, 2021; H. Wang & Wang, 2022; S. Wang & Wang, 2021).

This descriptive case study examines students' perceptions of developing an application prototype within a low-code platform. The prototype is one of the deliverables for the summative final project in a Systems Analysis and Design (SA&D) course. This paper begins with an overview of the related work, which informs the research question. The paper then describes the mixed methods approach to the descriptive research design. The qualitative data analysis utilized a thematic coding approach, while an intersectional analysis was performed on the quantitative data. Next the results are discussed in terms of how the students' perceptions of developing a low-code prototype answered the research question. The impact of the results is discussed, as well as the limitations of this study and avenues for future research, and finally, the conclusions are presented.

Background

Defining Low-Code

Low-code development platforms feature a Graphical User Interface (GUI), which allows the developer to easily drag widgets onto the screen for the application they are building and then quickly connect the widgets to code modules (Hyun, 2019). Applications featuring this type of modular design are easy to develop, very flexible, and straightforward to update (Hyun, 2019). The simplicity and ease of use inherent in low-code platforms allows expert users to develop applications without involving the IT department within their organization (Silva et al., 2020; Wild, 2021; Wolff, 2019). Low-code unlocks the potential for business users to develop their own apps, empowering more people to automate tasks through app development, and relieving some of the pressure from the shortage of professional software developers (Hirzel, 2022; Vikebø & Sydvold, 2019).

Use of Low-Code in Higher Education

Low-code tools are becoming increasingly popular tools for teaching within higher education (Lebens & Finnegan, 2021; S. Wang & Wang, 2021). Some low-code platforms, such as SAP's Mendix, include ready-made curriculum that allows faculty to more easily integrate these tools into their existing courses (Mew & Field, 2018). Preliminary research on the effectiveness of low-code tools in the classroom shows they are helpful for teaching app development to students without a programming background, such as business students (H. Wang & Wang, 2022).

Developing a Low-Code Project for a Systems Analysis and Design (SA&D) Course

The original project for the SA&D course did not involve prototyping, instead, students wrote a research paper about the steps in the Software Development LifeCycle (SDLC). For this research study, the SA&D project was re-designed to allow students to experience the steps of the SDLC instead of merely writing about them, with the goal of incorporating active learning by including activities like low-code prototyping. The foundation for active learning as a pedagogy is that learners should be active agents during instruction (Lombardi & Shipley, 2021). One of the tenets of active learning is that learners construct knowledge through action, not only through individual cognitive construction, but also through social construction by collaborating with other learners (Brame, 2016).

For the newly redesigned SA&D project, students choose a case that describes an information system that interests them. For example, a student might choose a case that describes how a retail company implemented a new Point-of-Sale (POS) system for a chain of clothing stores. Students exchange feedback on how well their case fits the project requirements via a peer review assignment and are allowed the opportunity to choose a new case if their classmates indicate the case would not fit the project requirements. After the student has finalized their case, the professor also approves the case to ensure it is appropriate for the course project.

Students then develop several deliverables based on the information system described in the case, including a project proposal, a budget, a Gantt chart showing the project tasks and timeline, a use case diagram, an activity diagram, and finally a prototype of the system interface. Students are provided with detailed project instructions, as shown in the Appendix. Each of the deliverables for the project is graded using a standard rubric, such as the rubric for the system prorotype that is included in the Appendix. The students receive the rubrics in advance, so that they can see how their project deliverables will be scored and also so they can use the rubric as a checklist to ensure they met all of the requirements for each project deliverable. The rubric for the low-code prototype portion of the project is included in the Appendix.

The project serves as a summative assessment for the course. During the course, students complete hands-on assignments which provide scaffolding for students to build their skills prior to embarking on the project. For example, students complete a homework assignment creating a Gantt chart in Microsoft Excel. This assignment allows students to practice developing a Gantt chart prior to having to do so for their project and also serves as a formative assessment of the student's ability. The goal is to foster the development of hands-on technical skills through active learning as a part of the formative assessments, and then to assess those technical skills using the project as a summative assessment.

The low-code environment chosen for the SA&D corse is Microsoft PowerApps. PowerApps features a GUI that allows students to easily create screens to mock up a system interface (Kumar, 2022). Students can drag-and-drop widgets, like buttons, textboxes, and menus, to quickly develop a prototype application (Kumar, 2022). Prior to developing the prototype of the system interface, students completed one of the official

Microsoft Learning Paths on creating apps with PowerApps. The Learning Paths serve as hands-on tutorials and are aimed at teaching IT professionals how to use specific Microsoft tools (Margo, 2022). The Learning Path for PowerApps is fairly in-depth and students create three different apps as a part of the Learning Path (Sarkar, 2022). The Learning Path serves as the formative assessment to prepare students for creating the prototype as a part of the course project.

Research Question

Based on the review of the literature, the following research question was developed:
What are students' perceptions of developing an application prototype using a low-code platform?

Methodology

This descriptive case study is a preliminary study intended to gather student perceptions of low-code prototyping in a single section of an SA&D course before initiating a larger-scale examination of students' experience with low-code prototyping over several sections of the same course. The aim of this case study is to establish if students perceive low-code prototyping positively enough to merit extending low-code prototyping to all sections of the SA&D course.

Research Design

The research method chosen for this study is a mixed methods approach, using an anonymous survey consisting of both open- and closed-ended questions. A mixed methods approach was chosen for this study because gathering qualitative data alongside of quantitative data provides a deeper perspective on students' perceptions that quantitative questions alone could provide. A research proposal was submitted to the Institutional Review Board (IRB) at the researcher's home institution and was approved prior to initiating the research. The students responding to the survey voluntarily agreed to a consent form which explained the risks and benefits of participating in the study prior to beginning the survey.

Research Setting and Participants

Low-code prototyping was introduced in the Spring 2022 semester of an undergraduate upper-division SA&D course for this study. This is a required course for Management Information Systems (MIS) majors. The sample consisted of thirty-two upper-division undergraduates enrolled in an online synchronous section of the SA&D course. Twenty of the thirty-two students in the sample participated in the study. The students are considered non-traditional students since the average age is 29 years old and the majority of students work full time. The research site is a public, urban university which is federally designated as a minority serving institution, since

the majority of students are students of color. Over 57% of enrolled students reside in lower-income households and 75% of are Pell grant eligible (Whelan, 2019; Wolfston, 2020).

Materials

A professional online survey tool, Qualtrics, was used to develop the survey instrument and gather the data. The survey instrument included the following questions.

- Q1: The low-code environment (PowerApps) was helpful in developing my prototype. Likert-Scale: Strongly agree, somewhat agree, neither agree nor disagree, somewhat disagree, strongly disagree
- Q2: I was able to develop my prototype quickly using the low-code environment (PowerApps). Likert-Scale: Strongly agree, somewhat agree, neither agree nor disagree, somewhat disagree, strongly disagree
- Q3: I would like to use a low-code environment in my current or future job. Likert-Scale: Strongly agree, somewhat agree, neither agree nor disagree, somewhat disagree, strongly disagree
- Q4: Prototyping using a low-code environment is a valuable experience to add to my resume. Likert-Scale: Strongly agree, somewhat agree, neither agree nor disagree, somewhat disagree, strongly disagree
- Q5: What are the benefits of using a low-code environment for prototyping? Open-ended response
- Q6: What are the drawbacks to using a low-code environment for prototyping? Open-ended response

Procedure

During an online Zoom class meeting, students were given ten minutes to complete the survey questions. The students' participation was entirely optional, and those students who declined to participate were provided the option to take a ten-minute break. No extra credit or other incentives were provided for participation.

Analysis

The quantitative data was analyzed using the data analytics tools within the Qualtrics platform and an intersectional analysis was performed. The researcher analyzed the qualitative data using an inductive coding analysis to identify frequent themes in the responses. Coding was utilized to develop codes to classify the responses to the survey questions. An inductive coding approach was chosen since the goal of this descriptive case study is to examine the students' open-ended observations on the benefits and drawbacks of low-code prototyping.

Results

Ninety percent of the students who responded to the first question (Q1) stated they strongly or somewhat agreed that the low-code environment (Microsoft PowerApps) was useful in developing their prototype. As shown in Figure 1, eleven respondents selected strongly agree for Q1, seven selected somewhat agree, one selected neither agree nor disagree, and one selected strongly disagree. Eighty percent of the students responding to Q2 strongly or somewhat agreed that they were able to develop their prototype quickly using the low-code environment. Eleven respondents selected strongly agree for Q2, five selected somewhat agree, two selected neither agree nor disagree, one selected somewhat disagree, and one selected strongly disagree, as shown in Figure 2.

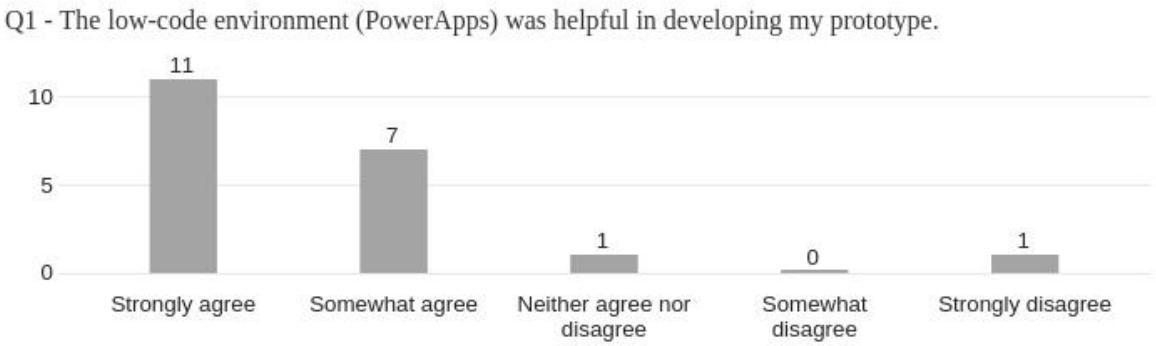

Figure 1. Responses to Survey Question 1

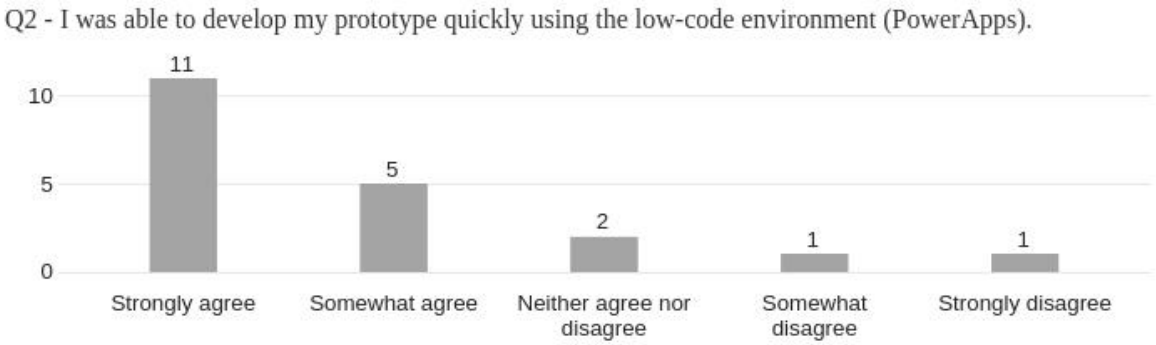

Figure 2. Responses to Survey Question 2

Eighty-five percent of the students indicated they strongly or somewhat agreed that they would like to use a low-code environment in their current or future job, in response to Q3. As shown in Figure 3, ten respondents strongly agreed, seven somewhat agreed, two neither agreed nor disagreed, and one strongly disagreed that they would like to use a low-code environment at their job. In response to Q4, eighty-five percent of students strongly or somewhat agreed that prototyping using a low-code environment was a valuable experience to add to their resume. Figure 4 shows the responses to Q4, with twelve respondents indicating they strongly agree, five

indicating they somewhat agree, one indicating that they neither agree nor disagree, one indicating that they slightly disagree, and one indicating that they strongly disagree.

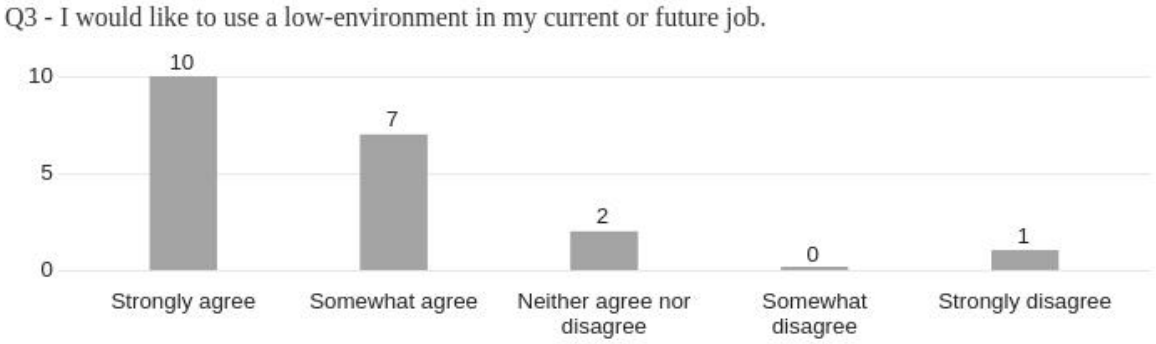

Figure 3. Responses to Survey Question 3

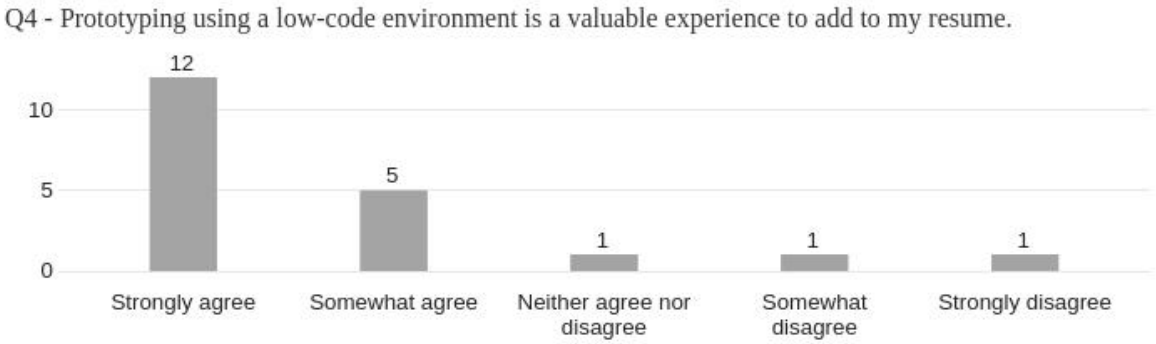

Figure 4. Responses to Survey Question 4

Inductive Coding Analysis

An inductive coding analysis was completed to examine the qualitative data and subsequently create codes based on the open-ended responses to Questions 5 and 6 (Q5 and Q6). While performing the inductive coding analysis, a number of common categories surfaced. These common categories were employed to assign codes to classify the responses to the survey questions. Comparable types of responses were grouped into categories in order to distinguish common themes. The full text of the responses to Q5 and Q6 is shown in the Appendix in Table 3 and can be matched by respondent ID to the codes shown in Tables 1 and 2.

Q5 asked students to describe the benefits of using a low-code environment for prototyping, while Q6 asked students to describe the drawbacks to using a low-code environment for prototyping. Common themes in the responses to Q5 were the low-code environment's ease-of-use and that no coding knowledge was required to build the prototype. Fifty-five percent of students described the low-code environment as easy to use, while fifty percent cited no required coding knowledge as a benefit. Students also describe using a visual prototype and the speed of development as benefits to the low-code environment. Table 1 shows how the responses from Q5 were coded.

Lack of coding skills and the limited features in the low-code environment were common themes in the responses to Q6 on the environment's drawbacks. Other drawbacks that were described by the students were lack of an auto-save feature in the low-code environment, difficulty using functions, and a large amount of content to read to learn how to use the environment. Three students stated there were no drawbacks and one did not give a response to Q6. Table 2 shows how the responses from Q6 were coded based on their content.

Table 1. Inductive Coding Analysis Results for Survey Question 5

Code	Respondent IDs
Ease of use	1,3,4,7,8,11,15,16,18,19,20
Features/tools	14
No coding knowledge required	2,5,6,7,10,12,13,16,17,18
Speed of development	7,11
Visual prototype	9,20

Table 2. Inductive Coding Analysis Results for Survey Question 6

Code	Respondent IDs
Functions difficult to use	14
Lack of auto-save feature	4
Lack of coding skills	1,10,13,15
Large amount of content to read	3
Limited features	5,6,7,8
None/Not applicable	2,11,12
No response	9

Discussion

The goal of this research was to discover students' perceptions of using a low-code environment to develop a prototype. At the outset of the newly developed SA&D course project, it was unknown if students would perceive the low-code environment as helpful or whether students would find the low-code environment difficult to use for prototyping, since it is a professional tool. Although students had the opportunity to practice using the low-code environment as a part of the Microsoft Learning Path assignment, it also seemed possible that this may not be adequate preparation for creating the prototype during the course project.

As it turned out, the students' perceptions of using the low-code environment for prototyping were mainly positive. The vast majority of students, ninety percent, agreed that the low-code environment was at least somewhat helpful in developing their prototype. Additionally, eighty percent of students agreed that they were able to create the prototype quickly using the low-code environment.

Students perceived the low-code environment as a tool they would like to use in their current or future career as an IT professional, with eighty five percent indicating they strongly or somewhat agree that they would like use low-code on the job. Eighty five percent of the students also feel experience with a low-code environment is a valuable addition to their resume. Based on the students' positive perceptions of the marketability of their low-code experience, it may make sense to advertise the SA&D course to students as a way to develop their low-code skills. Advertising to students that they will gain low-code experience may increase enrollment in the SA&D course.

The students' positive perceptions of the low-code environment dovetails with the literature showing the increasing popularity of low-code in higher education. Using the ready-made curriculum from Microsoft made it easier for faculty to integrate the low-code environment into the SA&D course, as students could easily access the Microsoft Learning Paths online for free. Although the students in this study were upper-division MIS majors, there was no programming or software development course as a pre-requisite to the SA&D course, as is typically required for MIS majors. Despite the lack of a software development course as a pre-requisite, students perceived the low-code environment as helpful and quick for developing their prototype. This reinforced the findings in the literature that low-code tools are helpful for teaching students who lack a programming background.

The qualitative portion of the study exposed more nuance in students' perceptions of the low-code environment. The slight majority of students described the low-code environment as easy to use. As one student stated, the low-code environment "makes it easier for a greenhorn to pick up and immediately start developing" (Respondent ID 5). Around half of the students described not needing to know how to code as a benefit of the environment. For example, one student described how the low-code environment "gives users the power to build an application for their business with minimal code knowledge" (Respondent ID 10).

Interestingly, while not needing to learn how to code was frequently described as a benefit of the low-code environment, it was also frequently described as a drawback. In this regard, student perceptions conflicted, with sometimes even the same respondent listing not needing code as both a benefit and a drawback. One student noted that the low-code environment "gives users an excuse to skimp on their code skills", while another said that "people will be less likely to want to learn how to code if they can just use low-code" (Respondent IDs 10 and 13). These responses seem to indicate that students place some value on gaining actual coding skills.

Not only did students perceive the lack of coding skills as a drawback to the low-code environment, they also perceived the limitations of the environment as a drawback as well. One student said that within the low-code environment "there can be restrictions that you didn't anticipate and can't as easily work around, as with code you have more creative freedom" (Respondent ID 7). Other students similarly described this sense of restrictions within the low-code environment, with one student noting that "you may be limited in what you can do" within the low-code environment, while another simply said, "I feel as though there is more you can do with actual coding" (Respondent IDs 5 and 6).

While it was clear from reviewing the literature that low-code is becoming increasingly popular within both business and higher education, the results of this study show that students perceive the limits of the low-code environment along with the lack of coding skills as drawbacks. This indicates that faculty should consider not replacing software development or coding with low-code, but instead use a low-code environment to supplement what students are already learning in a more traditional programming course. In this regard, the low-code environment fits well as a tool for prototyping in the SA&D course, since low-code is not being used as a substitute for learning how to code in this particular course. Instead, the low-code environment gives students an opportunity to apply what they have learned about the SDLC by developing a system prorotype.

Limitations and Future Research

A key limitation of this case study is the sample size, since the scope was limited to information systems students in a single section of an SA&D course. The intent of this descriptive case study is to serve as preliminary research to spur a larger longitudinal study. The results of this case study will be utilized to refine the summative project for the SA&D course. Future research will be conducted in subsequent terms to gather additional data on students' perceptions of prototyping using a low-code environment.

Conclusion

While students' perceptions of low-code prototyping were primarily positive, with most students agreeing that low-code environment was easy-to-use for prototype development, student also identified key drawbacks to using low-code. Students perceived the lack of learning coding skills and the limitations of the low-code environment as distinct drawbacks. The broad implication of these findings is that although low-code environments are effective for creating prototypes, faculty should exercise caution when replacing conventional coding skills solely with low-code skills. This study takes place at a crucial time, due to the skyrocketing popularity of low-code environments within both business and higher education. The results of this study, coupled with the project instructions and rubrics provided in this paper, provide faculty with the momentum and tools to implement low-code prototyping in their own courses. The broad impact of this study is that it provides a working proof-of-concept and starting point for faculty involved in developing low-code course curriculum.

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Appendix. Instructions for Course Project

Deliverable 1: Choose a Case

You have the opportunity to choose your own case for this project. When choosing your case, keep in mind that it must describe an information system and be substantial enough to meet the objectives described in the project instructions. Your choice of case is subject to the approval of the instructor, in order to ensure the case will work well for you as you progress through the project.

Deliverable 2: Case Feedback

1. Log into the Case Review discussion board.
2. Choose two cases to review for your classmates.
3. Answer the following questions for each of the cases:
    a. Does the case meet the requirements of the systems analysis project, as described in the Systems Analysis Project Instructions? Why or why not?
    b. Is it possible to write a proposal for the system described in the case that meets the requirements in Systems Analysis Project Instructions? Why or why not?
    c. Do you think your classmate will be able to create the diagrams to model the system described in the case, based on the project instructions? Why or why not?
    d. Can the system or a part of the system described in the case be modeled as a prototype?
    e. What is your general feedback for your classmates on the cases they have chosen?
4. Post your answers in the Case Review discussion board to share your feedback with your classmates.

Deliverable 3: Project Proposal

1. Executive Summary

Describe the purpose of your system and the audience for your system. Imagine you are writing for top management, such as the CEO of your company. Be persuasive as to why this system is a worthwhile endeavor for your company to undertake.

2. Description of Users and Development Approach

2.1 Users

- Make a list of the users of the system.
- Who will be using the system once it is implemented? Are all of the users described in the case? Are there potential users who are not mentioned in the case?

2.2. Development Approach

- Recommend a development approach for the project, either using the traditional waterfall approach or an Agile approach.
- Justify why the approach you chose is optimal for this systems project.

3. System Requirements

- Define the requirements for the system, both functional and non-functional. The requirements describe what the system will do.

3.1 Functional Requirements

- Describe the functional requirements for the system using user stories. You need to include at least ten user stories that describe the functional requirements for the system.

3.2 Non-Functional Requirements

- Describe the non-functional requirements for the system. The non-functional requirements should include the following requirements:
    - Technical
    - Performance
    - Usability
    - Reliability
    - Security

4. Budget

- Create an estimated budget for the project.
- Use the system requirements to help you decide what line items to include in the budget.

5. Gantt-lite Chart

- Develop a Gantt-lite chart using the description in your textbook.
- Each task in the Gantt-lite chart should be assigned a deadline.

Deliverable 4: Proposal Review

You will have the chance to participate in a review of your proposal to garner valuable feedback from your classmates.

1. A rough draft of your proposal is due by the due date listed on the course schedule. Share your rough draft with your classmates to get feedback by posting it on the discussion board.
2. Download at least two classmates' proposals. Add your feedback to your classmates' proposals using the "track changes" and comments features in Word.
3. Post the copies of your classmates' proposals containing your feedback to the discussion board to share your feedback.

4. After the review session, use your classmates' feedback revise your proposal.
5. Upload your final proposal to the appropriate drop box by the due date listed on the course schedule.

Deliverable 5: System Diagrams

1. Make a list of five or more major use cases for the system.
    - A use case describes one of the activities that the system performs in response to a user request.
    - You can identify the major use cases by analyzing the business processes.
2. Of the possible use cases you have identified, choose the most crucial use case to the functioning of the system. Write a use case narrative describing that use case. There are three levels of detail for use cases: casual, brief, and fully dressed. This use case narrative should be written at the fully dressed level.
3. Create a use case diagram for the use case you identified as crucial to the functioning of the system.
4. Create an activity diagram for the use case you identified as crucial to the functioning of the system.

Deliverable 6: System Diagrams Review

Similar to the proposal review, you will have the chance to participate in a review of your system diagrams to garner valuable feedback from your classmates.

Directions for Review

1. A rough draft of your systems diagrams is due by the due date listed on the course schedule. Share the rough draft of your diagrams with your classmates to get feedback by posting the diagrams on the discussion board.
2. Download at least two of your classmates' diagrams. Post your feedback on your classmates' diagrams on the discussion board.
3. After the review session, revise your diagrams based on your classmates' feedback.
4. Upload your final proposal containing your diagrams to the appropriate drop box by the due date listed on the course schedule.

Deliverable 7: App Prototype

1. Use PowerApps to develop an app prototype for the user interface for the system.
2. Employ the principles of user-centered design to create the user interface.
3. The app prototype should represent what is described in your requirements in the proposal document.
4. Remember that the prototype does not need to be fully functional, instead, a prototype gives the client a chance to see an interactive example of the User Interface (UI).
5. Be prepared to discuss what happened while developing the prototype in your retrospective and describe any changes that were made to the requirements document. Although it is challenging when you are not

able to meet the original requirements, it is a wonderful opportunity for learning and experiencing what it is like to be on a development project first-hand.

6. Test your app prototype to ensure it works according to your specifications in the requirements document.
7. Export your app to a zip file and upload it to the appropriate drop box.

Deliverable 8: Retrospective

Purpose of the Retrospective:

Near the end of the course, you will develop a retrospective that documents your systems analysis project. Project retrospectives help teams examine what went well and what went wrong on a project. The term "retrospective" comes from the Agile project methodology. A retrospective is reflecting on the past to improve the future. During this retrospective, you will identify on how to improve by reflecting on what worked, what didn't, and why.

Why do we have a retrospective as a part of this class? The goal of the retrospective is to reflect on what went well during the project, as well as the low points of the project, to improve on future projects. ? Retrospectives are a critical practice of the Agile methodology. The retrospective gives you the opportunity to share your work with your peers and gain valuable experience in critiquing your own and other students' work, which sharpens your critical thinking and communication skills. Finally, your retrospective can be added to your portfolio to share with potential employers.

Instructions for the Retrospective Video:

1. Format

Record a short video for your retrospective.

2. Timing

The presentation length should be no longer than 10 minutes.

3. Content

Your retrospective will contain two parts. In the first half of the retrospective, you will give a short overview of the project plan and the app. Then in the second half of the video, you will reflect on the process of developing the app together. In the Agile methodology, this is called a "retrospective". The retrospective helps developers think about how they can improve future projects. During the retrospective you discuss the high points, challenges, and lessons learned from this project. The retrospective is the most important part of the retrospective, because the lessons learned from this project will impact your success on future projects, whether in college or on the job.

Retrospective Outline:

Part 1: Project Plan, System Diagrams, and App Prototype

1. Why did you choose this case?
2. Why is it important to you to develop this system?

3. Give a very brief overview of the proposal, just enough to familiarize the audience with the planned functionality for the system.
4. Share your system diagrams.
5. Give a short demonstration of the app prototype.
6. Answer the following questions:
    a. Did the app protoype development process go as planned in your project plan? Why or why not?
    b. How well did the prototype meet the original requirements?
    c. What types of changes needed to be made to the requirements as the prototype was being developed?

Part 2: Reflection

1. Describe what went well on the project. What were the high points?
2. Describe the challenges and issues you encountered during the project.
3. Explain how you overcame the challenges you encountered during the project.
4. Describe what would you do differently if you had the project to do over again. What are your takeaways or lessons learned from this project?

Sharing your Retrospective:

1. Please upload your video to the course website.
2. Post the web link to your video on the retrospectives discussion board to share the link with classmates.
3. Upload a Word document containing the link to your video to share it with your instructor for grading.

Giving Feedback on Retrospectives:

1. Watch your classmates' retrospectives on the retrospectives discussion board.
2. Post feedback on at least two classmates' retrospectives on the retrospectives discussion board.

Rubric for Low-Code Prototype

Criteria	Professional	Amateur
Prototype - User Interface	15 points The user interface for the prototype was appropriate for the system, based on the description of the system given in the project proposal.	10 points The user interface for the prototype was mostly appropriate for the system, based on the description of the system given in the project proposal. One to two elements were missing or did not fit with the description in the proposal.
User-Centered Design	5 points The web prototype employed the principles of user-centered design.	3 points The web prototype mostly employed the principles of user-centered design. One or two elements were not user-friendly.

Figure 5. Rubric for Low-Code Prototype – Professional and Amateur Levels

Unsatisfactory	Incomplete	Criterion Score
0 points The user interface for the web prototype was not appropriate for the system, based on the description of the system given in the project proposal. Multiple elements were missing or did not fit with the description in the proposal.	0 points The zip file containing the prototype was not uploaded to the dropbox by the due date on the course schedule.	/ 15
0 points The link to the web prototype was not posted to the dropbox by the due date OR The web prototype did not employ the principles of user-centered design. Multiple elements were not user-friendly.	0 points The zip file containing the prototype was not uploaded to the dropbox by the due date on the course schedule.	/ 5

Figure 6. Rubric for Low-Code Prototype – Unsatisfactory and Incomplete Levels

Responses to Open-Ended Survey Questions

Table 3. Responses to Open-Ended Survey Questions

Respondent ID	Survey Question 5: What are the benefits of using a low-code environment for prototyping?	Survey Question 6: What are the drawbacks to using a low-code environment for prototyping?
1	Ease of use and efficiency.	Lack of understanding of coding
2	It help [*sic*] me create an app without coding	I don't know
3	its [*sic*] easy to understand.	It has a lot of content to read
4	easy to create prototype from scratch	don't save the work automatically.
5	It makes it easier for a greenhorn to pick up and immediately start developing.	You may be limited in what you can do. It is more beginner-friendly, yes, but begins to limit what more advanced users can do.
6	You do not need to know how to write code in order to build an app	I feel as though there is more that you can do with actual coding
7	I'm able to do things that I couldn't do as quickly as I could if I was to code it. I love the ease and convenience of it.	There can be restrictions that you didn't anticipate and can't as easily work around, as with code you have more creative freedom.
8	Easy to prototype for new people	Some complicated prototyping features not yet available
9	It is pretty simple and helps you see what you are trying to make. Sort of like a rough draft.	[No response]
10	gives users the power to build an application for their business with minimal code knowledge	it gives users an excuse to skimp on their code skills
11	Easy to learn. Can turn a spreadsheet into a functioning app in about 5 minutes.	None.
12	Great for non-experience and beginning professionals.	NA
13	Helping for people who don't have any experiences in coding.	People will be less likely to want to learn how to code if they can just use low-code
14	There are tons of tools that you can use.	Coming up with functions was difficult for me.
15	The benefits of using a low-code was it is something you can easily follow along.	The drawbacks were how to develop the code and make it run
16	It makes it easier for a greenhorn to pick up and immediately start developing.	[No response]
17	You do not need to know how to write code in order to build an app	[No response]

18	I'm able to do things that I couldn't do as quickly as I could if I was to code it. I love the ease and convenience of it.	[No response]
19	Easy to prototype for new people	[No response]
20	It is pretty simple and helps you see what you are trying to make. Sort of like a rough draft.	[No response]

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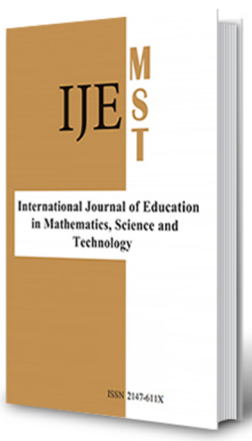

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