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## Full issue– Volume 3 Issue 1, 2020

### Cover Page Footnote

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**PURSUE: Undergraduate Research Journal**

**Volume 3 Issue 1, 2020**

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### **A Note from the Executive Editor**

It is with great pleasure that I present to you Volume 3 Issue 1 of *PURSUE*. All articles in this issue are peer reviewed by scientists. The peer review process is a competitive one. Only those articles recommended by both the review editors, who provide discipline specific expertise, and the editorial board were selected. This issue includes insightful articles in the areas of agriculture, biological engineering, health and nutrition.

The entire Editorial Board and Review Editors must be thanked for this issue. They provided countless hours selecting review editors, reviewing manuscripts, formatting documents proofing, and creating our new graphic design for the Digital Commons platform.

This is the first issue of the journal housed on the PVAMU Digital Commons platform. This platform exposes the research articles to a wider audience. A special thanks goes to the PVAMU library staff for facilitating the journal's online move.

Happy Scientific Reading!

Yolander R. Youngblood, Ph.D.  
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PURSUE  
Asst. Professor of Biology  
Prairie View A & M University

**Harvesting Electrical Energy Produced by Electrogenic Bacteria in Microbial Fuel Cells**

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## Abstract

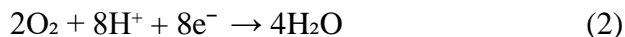
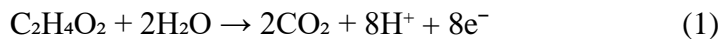
Cellular respiration is the process by which organic matter oxidizes, and the energy stored in the chemical bonds of the food releases. Normally, cellular respiration occurs inside the mitochondria of cells; however, a unique type of bacteria releases electrons externally. These specialized organisms are called electrogenic bacteria. Our goal is to construct a microbial fuel cell (MFC) with electrogenic bacteria, harvest the external electrons created by cellular respiration, and channel them through an external circuit to generate electricity. Mud soil, which has a high number of electrogenic bacteria in the environment, was used to construct an MFC. In the presence of gram-negative bacteria, which exist in both aerobic and anaerobic conditions, the constructed MFC delivered electrical energy to an external circuit. The MFC can generate electricity, and thereby power, from biodegradable substances and organic wastes found in the environment and landfills. They can also be used to power small devices and sensors used in day-to-day activities. To determine the effect of sugar on the growth and development of bacteria present in the MFC, the quantity of sugar administered will be monitored in relation to the power generated per day.

**Keywords:** Power generation, Electrogenic bacteria, Bioenergy, Microbes, Microbial Fuel Cell.

## Introduction

With an increase in the generation of organic waste and the constant need for electricity in day-to-day activities, the use of microbial fuel cells (MFCs) is gaining momentum. MFCs can be used to treat wastewater and generate electricity from organic waste (Mercer, 2010). Also known as the redox reaction, two key reactions occur in MFCs: 1) reduction of hydrogen and 2) oxidation (loss of electrons); they occur in separate regions of the fuel cell (Rozendal, Hamelers, Rabaey, Keller, & Buisman, 2008). Also known as biological fuel cells, MFCs can drive current using microbes/bacteria found in the environment in their natural state. In MFCs, microorganisms undergo a major bioelectrochemical reaction, which converts the mass of organisms in each area into electricity or hydrogen/chemical products (Pant et al., 2012). Ideally, an MFC consists of a cathode, where hydrogen ions generated by the microbes interact with the electrons and undergo reduction and an anode where oxidation occurs. The cathode and anode are typically separated by a proton exchange membrane, such as porous mud (Ghasemi et al., 2012). The organic electron donor is present in the anode chamber, where oxidation takes place (Liu & Logan, 2004). During the generation of electrons by the microbes, a biofilm is developed around the anode, and this is spurred by the growth of cells on the surface of the electrode (Gottenbos, Vander Mei, & Busscher, 1999). The biofilm found in MFCs accommodates microbes, which allows free electrons to be transmitted to the anode. For an MFC to function effectively, electrons generated by the bacteria in the anode chamber must flow through a wire to the cathode where it reacts with oxygen from the exposed cathode (Lohner & Rowland, 2016) for continuous current. The electron donor produces CO<sub>2</sub>, protons, and electrons when oxidized, as seen in equation 1. The protons produced at the anode pass through the proton exchange membrane to the cathode (Rahimnejad, Najafpour & Ghoreyshi, 2011), and the reaction of

protons and electrons at the cathode in the presence of oxygen results in the formation of water as seen in equation 2. Based on the level of energy generated, the MFC can be renewable sources of energy for small devices such as biosensors (Rahimnejad, Adhami, Darvari, Zirepour, & Oh, 2015).



The MFC has two halves: aerobic and anaerobic (Mercer, 2010). The aerobic chamber has a positively charged electrode and is oxygenated. The anaerobic chamber does not have oxygen, thereby enabling a negatively charged electrode to serve as the electron acceptor for the bacterial process.

There are two types of MFCs: mediated and mediator-free (Huang, Zeng, & Angelidaki, 2008).

- Mediated MFCs: The mediators in these MFCs spur or facilitate the movement of electrons to the electrode from the microbial cells (Delaney et al., 1984/2008). Examples of such mediators are thionine, methylene blue, and benzyl viologen (Roller et al., 1984). Mediated MFCs mainly utilize electrochemically inactive microbial cells.
- Mediator-free MFCs: In this case, electrons move to the electrodes via electrochemically active bacteria. Examples of such bacteria are *Shewanella sp* (Kim, Kim, Hyun, & Park, 1999a) and *Geobacteraceae* (Bond & Lovley, 2003).

The kind of electron transfer mediator and the bacteria used determine the efficiency of an MFC, which is often measured by the quantity of oxygen consumed (Roller et al., 1984). Thus, the MFC needs to operate at a pH close to 7 and a temperature between 68°F and 104°F (20°C and 40°C) (Bullen, Arnot, Lakeman, & Walsh, 2006).

*Shewanella sp.* is gram-negative bacteria that can respire in both aerobic and anaerobic environments (Nordberg et al., 2014). They possess thread-like structures known as flagella that enable motility and aid in generating and passing of electrons. *Shewanella* appears rod-like, as seen in Figure 1A. *Shewanella* form a biofilm on the anode in which they stick together (Hall-Stoodley, Costerton, & Stoodley, 2004) and produce protons. This biofilm helps decompose acetate to generate electricity (Reguera et al., 2006).

*Geobacter sp.* live in anaerobic conditions, which has made them relevant in the bioremediation of organic compounds (Childers, 2002) and the production of electricity. They are gram-negative bacteria that generate electricity by oxidizing compounds and reducing the anode where they are attached. *Geobacter* have long nanowires known as pili--extracellular tubules believed to conduct the flow of electrons. The high level of electron transfer via the pili encourages the collation of *Geobacter* at the anode, the formation of a thick biofilm, and the generation of current (Reguera et al., 2006). The pili can grow up to 20 micrometers (Strycharz-Claven, Snider, Guiseppi-Eliec, & Tender, 2011). Figure 1B shows the nature of a *Geobacter*.



Figure 1A: *Shewanella sp.* all connected  
with the flagella

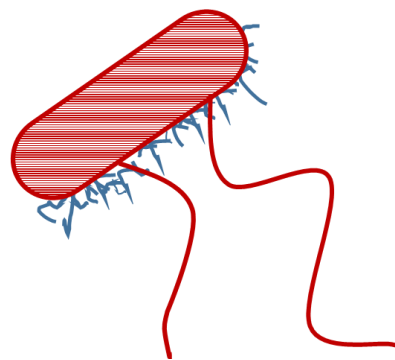


Figure 1B: *Geobacter sp.* with the pili

Due to *Shewanella sp.*'s respiration mode, there can be a correlation between the electricity generation and the growth of the bacteria on the electrodes. In our experiments, we have tried to quantify that effect. It is important to note that both bacteria possess flagella/pili, which aid in the generation and transmission of electrons. Mud soil may naturally have a high number of microbes in the environment; therefore, mud soil has the potential for high energy generation. With this in view, we used mud soil collected from a field beside a poultry farm to create a model MFC system and test the hypothesis that energy generation will increase when table sugar is present with the MFC's microbes.

## Materials and Method

Various structures can improve the performance of MFCs (Du, Li, & Gu, 2007). The optimal design is necessary for maximum efficiency and power generation. Two identical MFCs were assembled, one to serve as a control, which has no treatment after setting up, and the other was supplied with sugar as a food source. The number of bacteria were measured at the inception of the project and when sugar was added to one MFC, and the power output was compared with the control.

### MFC Preparation

A Sieve #18 with a 1.00 mm opening and 0.0394 inches was used to strain the soil. Small, hard particles such as rocks and pebbles were removed. These particles are removed to reduce the chances of aeration within the soil, particularly for bacteria which operate in anaerobic condition. Two teaspoons full of the soil were scooped into a beaker and set aside after the soil was prepared. This beaker of soil is used to measure the initial quantity of bacteria in the soil (See Bacteria Count Section). After preparing the soil, the MFCs were assembled and labeled as "control" or "sugar." The MFC units are filled with soil up to the point marking 1 centimeter on the unit and patted to give the soil a smooth surface. It is important to make a smooth surface to avoid any form of aeration which might affect the bacteria within the anode compartment. The anode was placed on and pressed against the soil to remove air bubbles. A wire was connected to the anode and stretched on the side of the unit. The MFC is then filled



with more soil up to the 5-centimeter mark and patted to get a smooth surface. The cathode, which is a thicker, black graphite foam with another wire connected to it, was placed on the soil. It is important to avoid any form of liquid or soil to cover the top of the cathode as it might affect the conversion of oxygen to water. The cathode wire was placed into the “+” port of the hacker board (MudWatt Inc.), and the anode wire was placed into the “-” port of the same. A 10 $\mu$ F 50 V capacitor and a LED were then plugged into the ports of the hacker board. It is important that the capacitor and LED are inserted the right way to avoid any form of interference.

### **Bacteria Count**

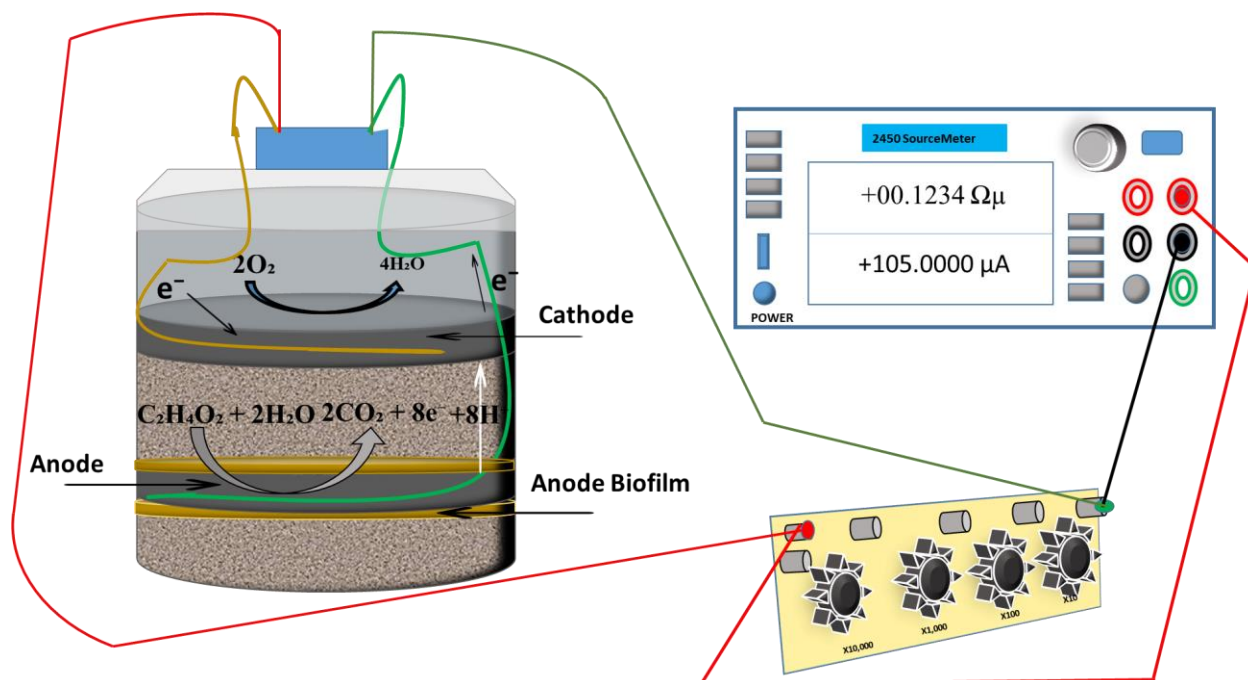
Comparing the total number of cells present in the MFCs at the inception of the project to the number present at the time sugar is added gives a better understanding of how the cell count affects the level of power generated by the MFCs. After setting up the MFCs, the beaker of soil that was collected was used for the initial bacteria count. Three beakers are prepared and labeled A, B, and C for a serial dilution factor of 10<sup>-2</sup>, 10<sup>-4</sup> and, 10<sup>-6</sup>, respectively. A balance (Ohaus Adventurer AR3130) was used to measure 0.5 g of soil that was placed in Beaker A; each beaker was then filled with 49.5 mL of distilled water. Beaker A was stirred thoroughly with a sterilized spoon. Afterward, 0.5 mL of the soil-water mixture from Beaker A was aspirated with a pipette and added to Beaker B, which was then mixed with a sterilized spoon. Beaker C received 0.5 mL of the solution from Beaker B and was stirred. A sample (0.5 mL) was drawn from each beaker and dropped on the surface of a labeled nutrient agar plate and spread over the surface of the agar until the surface appears dry. The lid is closed, and the plates are then inverted and incubated at 85°F (29.44°C) for 24 hours in an incubator (Quincy Lab Inc., Model 10-140). Sugar was added to one MFC (See Power Generation Section), and another bacteria count was done, following the same process. However, a more diluted sample was needed because of the increased number of bacteria. Dilutions of 10<sup>-6</sup>, and 10<sup>-8</sup> were prepared for these experiments.

### **Electricity Generation in MFCs**

The LED connected to the external surface of the MFC starts to blink once the MFC generates electrical energy. The rate of blinking served as a measurement of power generated. The time difference between each blink was tracked with a stopwatch and recorded.

### **Measuring Power Generated**

The voltage generated by the MFCs was measured daily to check the power generated. Measuring voltage generated from a microbial fuel cell requires a connection to the hacker board and special configuration. The LED and capacitor were removed from the board, and a WPA N73 Resistance Box Voltage Divider was plugged into the circuit to measure the potential drop across the external resistance. For this experiment, seven resistors were used: 4700  $\Omega$ , 2200  $\Omega$ , 1000  $\Omega$ , 470  $\Omega$ , 220  $\Omega$ , 100  $\Omega$ , and 50  $\Omega$ . When plugged, the resistor was left on for at least five minutes before the voltage generated is measured by a multimeter (Keithley 2450 SourceMeter, Tektronix, Inc, Beaverton, Oregon). The voltage was checked for each of the resistors on both MFCs. After the voltage was measured, the LED and 10 $\mu$ F 50 V capacitor were placed back into the hacker board. Figure 2 shows the schematic of the whole MFC set up.



**Figure 2:** Schematic of the microbial fuel cell

### Power Generation

As the level of power generation from both MFCs stabilized, table sugar was added to the MFC labeled “Sugar.” The addition of sugar to the fuel cell was completed as follows. Table sugar (0.5 g) was added to a beaker of 10 mL of distilled water. All the cables were disconnected from the hacker board, and the lid was opened. A teaspoon scooped ~1 cm of soil into the unit. A transfer pipette was used to spread all of the sugar solution (10mL) on top of and mixed into the soil. After ~5 minutes, the MFC was reassembled. At the same time a soil sample is taken for a bacterial count using the same methods as stated above. After five days, another 0.5 g of table sugar is added to the MFC. From the decline in the number of blinks observed on the LED and the voltage measured, another 0.5 g of sugar would be added to the MFC after seven days.

## Results

### Cell Count for MFC

To determine the number of cells present, we counted the number of clusters present on the nutrient agar plates after incubation for 24 hours. The control plate with the  $10^{-4}$  dilution had 147 cell clusters (Figure 3A), and the control plate with the  $10^{-6}$  dilution had 128 cell clusters (Figure 3B). The second round of cell counting was completed after the addition of distilled water and/or table sugar and is detailed in Table 1. The lower solution shows a significant reduction in the number of clusters, this is a result of the dilution from preceding plates as seen in Figure 4C and 4D.



Figure 3A: Nutrient agar with the  $10^{-4}$  dilution of the initial soil

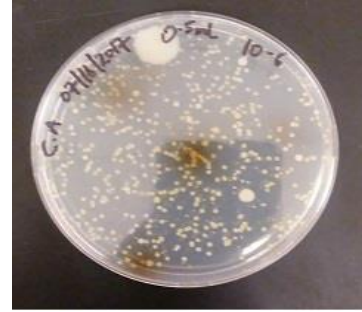


Figure 3B: Nutrient agar with the  $10^{-6}$  dilution of the initial soil

<b>Table 1: Cell counts based on the dilution factor for each MFC.</b>		
Dilution	Number of Cell Clusters for MFC (Control)	Number of Cell Clusters for MFC (Sugar)
$10^{-6}$	49	30
$10^{-8}$	25	27

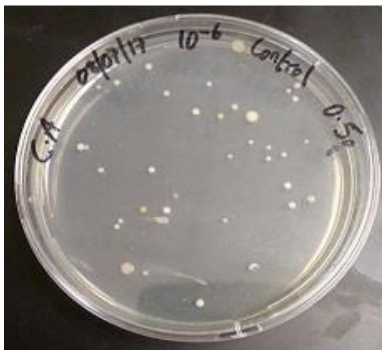


Figure 4A: Nutrient agar with the  $10^{-6}$  dilution for control MFC



Figure 4B: Nutrient agar with the  $10^{-6}$  dilution for sugar MFC



Figure 4C: Nutrient agar with the  $10^{-8}$  dilution for control MFC



Figure 4D: Nutrient agar with the  $10^{-8}$  dilution for sugar MFC

## Power Output from MFCs

The voltage measured across each resistor was tabulated for each day as shown in Table 2. The power generated was then calculated from Ohm's law, as shown in equation 3.

$$P = V^2/R \quad (3)$$

Where P is power in Watt (W)

V is the voltage in volts (V)

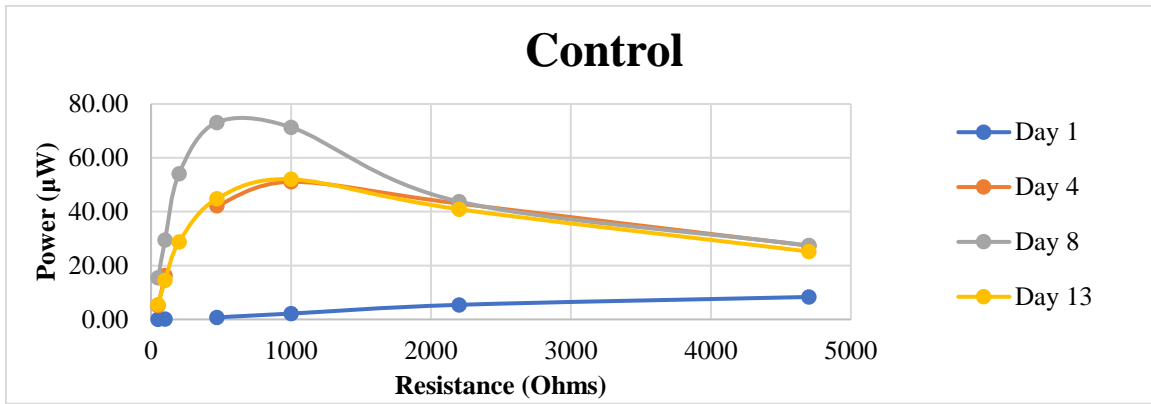
R is resistance in ohms ( $\Omega$ )

With the use of several resistors, a curve is generated, which shows the relationship between the power and the resistance for each MFC as seen in Figure 5. This also exhibits the level of power generated by the microbes in each MFC.

Resistance ( $\Omega$ )	Control		Sugar	
	Voltage (mV)	Power ( $\mu$ W)	Voltage (mV)	Power ( $\mu$ W)
4700	278.700	16.53	134.567	3.85
2200	256.900	30.00	119.947	6.54
1000	206.400	42.60	91.567	8.39
470	134.625	38.56	52.213	5.80
220	47.510	13.93	28.751	3.76
100	37.325	10.26	13.217	1.75
50	18.127	6.57	6.513	0.85

By the end of the thirteenth day of voltage measurement, the power-resistance curve showed some stability in the power generated. The peak power was not more or less than 10% different on days 11, 12 and 13 (Figure 5A and 5B). With this observation, 10 mL of 0.5 g diluted table sugar was introduced to the MFC labeled "Sugar," and 10 mL of distilled water was introduced to the MFC labeled "Control." Figure 6 shows the power-resistance curves after sugar was added. There was an increase in the number of volts generated for two consecutive days in each MFC after the addition of water/sugar. However, by Day 16, the number of volts generated by the MFC with sugar reduced gradually. By Day 18, the maximum power generated by the sugar MFC was 50% less than that the power generated on Day 15.

A.



B.

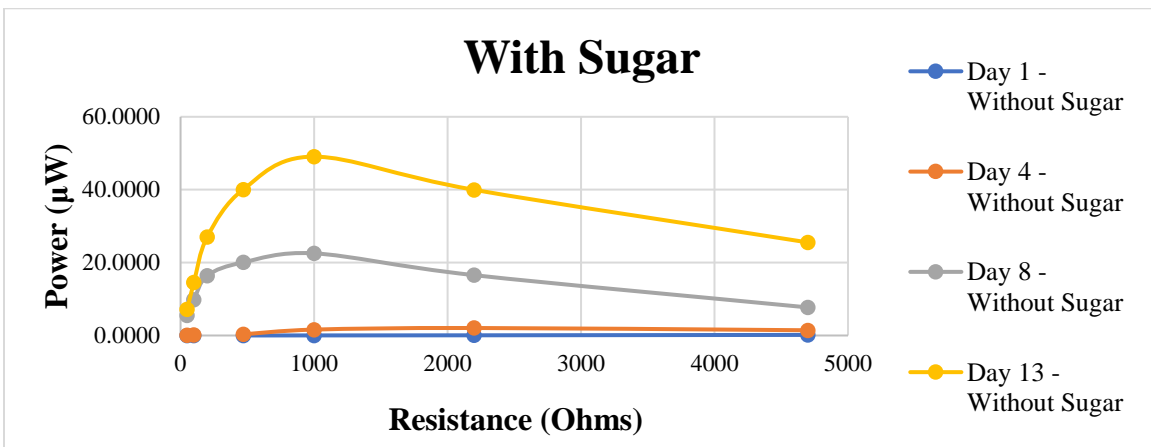


Figure 5: Curves showing the relationship between power and resistance for each microbial fuel cell from Day 1 to Day 13.

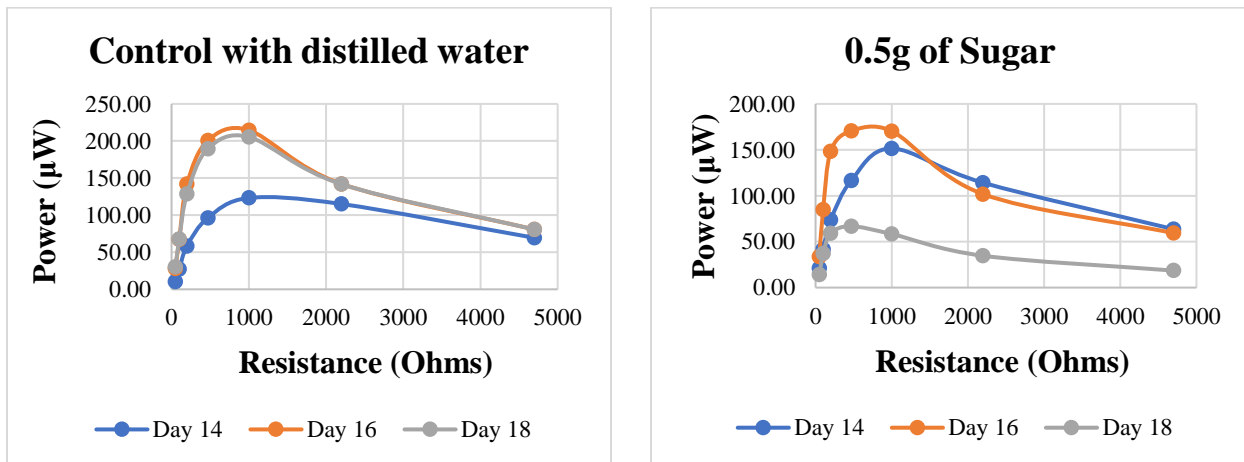


Figure 6: Curves showing the relationship between power and resistance for each microbial fuel cell after 10 mL of distilled water was added to control MFC and 10 mL of 0.5 g table sugar was added to the sugar MFC.

Investigators wanted to determine how fast the quantity of sugar introduced is consumed and metabolized (Figure 7). On Day 19, 10 mL of 0.5 g of table sugar or distilled water were added to appropriate MFC. Once again, an increase in the maximum power generated was observed. The Control MFC generated only slightly higher power, but the Sugar MFC had a 73% increase in the maximum power generated. By Day 22, the maximum power generated by the Sugar MFC began to subside. Figure 8 compares the maximum power generated per day for each MFC.

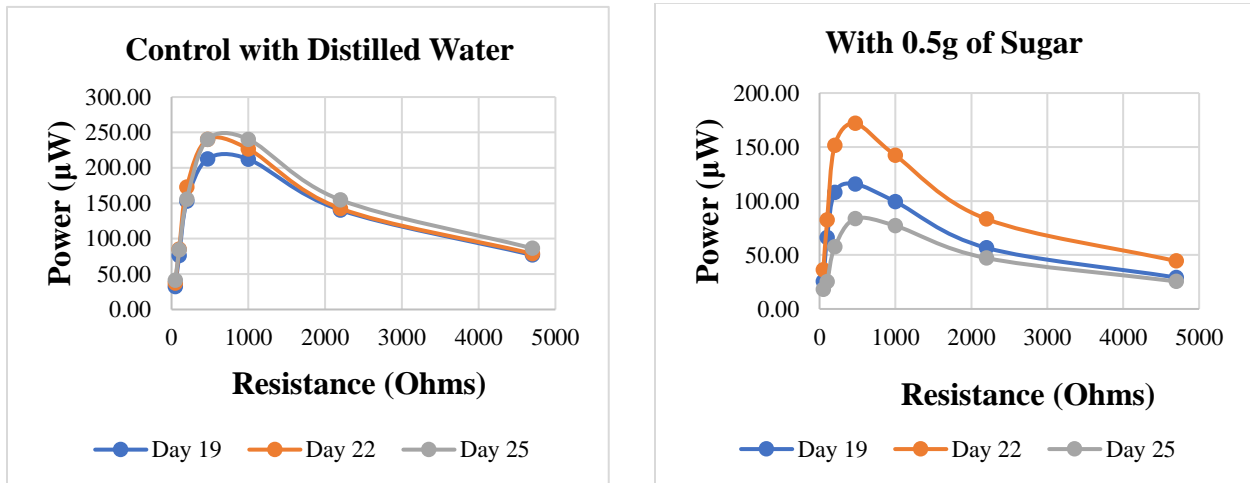


Figure 7: Curves showing the relationship between power and resistance for each microbial fuel cell after 10 mL of distilled water was added to the control MFC and 10 mL of 0.5 g table sugar was added to the sugar MFC.

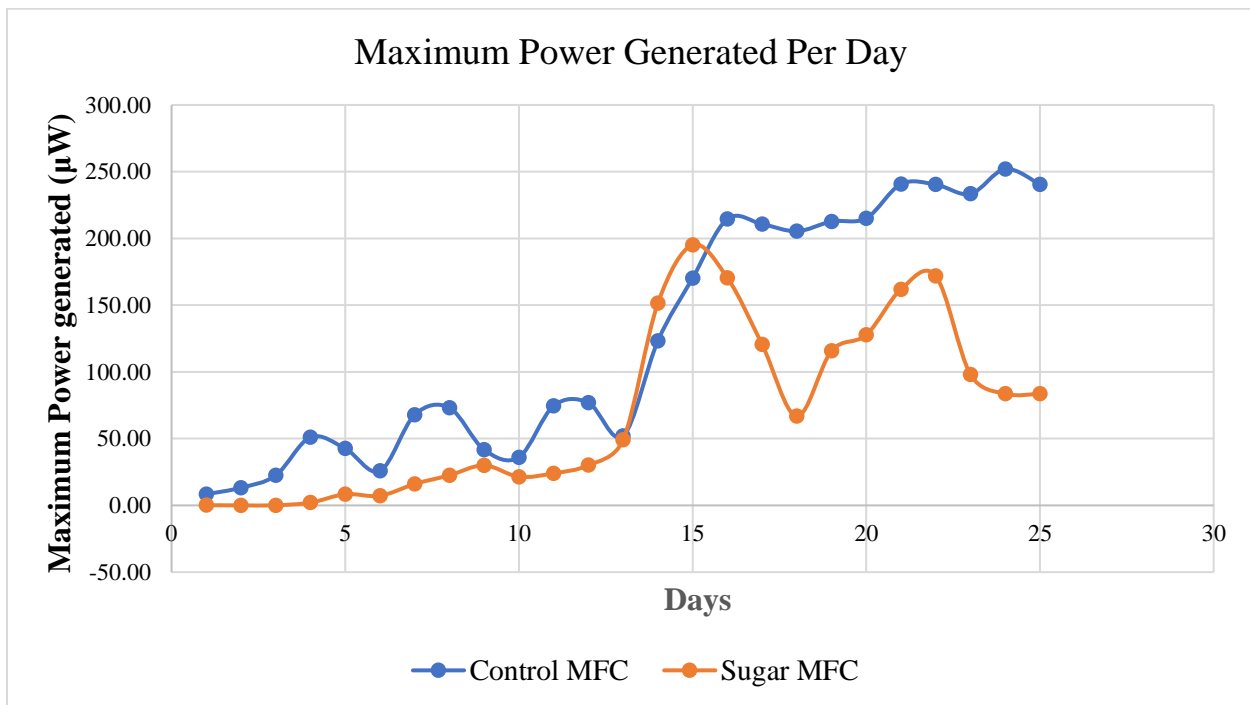


Figure 8: Maximum power generated per day for each MFC.

## Discussion and Conclusion

From the results obtained, the power generated increases soon after the introduction of table sugar (glucose). However, the power lasts for only a few days. The inhibition effect occurs because of voltammetry. Glucose was fed directly into the soil, and the time of degradation of the sugar increased in each case as a result of the reduction in the amount of oxygen present within the MFC. This is most likely due to the bacteria directly consuming oxygen, which flows to the anode as an electron acceptor. Ideally, the anode bacteria use oxygen around the anode to generate electrons which are passed from the anode chamber to the cathode chamber via the connecting wire. However, with the sugar introduced into the MFC, the protons are passed freely to the cathode through the soil separating both chambers along with the protons passed through the wire. The low energy produced might be a result of the fermentation of glucose, which cannot produce electricity. Methanogenesis occurs during the anaerobic respiration of *Geobacter sp.*, which hampers the growth of the bacteria. Rabaey, Lissens, Siciliano and Verstraete (2003) built an MFC which worked on generating more power with glucose, but that was after making it anoxic before administration. Sugar should be used to generate more power in MFCs and if a *Geobacter* is used, attention should be paid to making the MFC an anoxic environment to reduce the toxic effect on the *Geobacter species*.

## Acknowledgment

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## **The Effects of Fertilizer Rate on the Growth of Egyptian Spinach in a Greenhouse**

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## Abstract

Egyptian spinach (*Corchorus olitorius*, L.) is an annual herb and a popular vegetable grown in the dry, semi-arid and humid regions of Africa. It belongs to the American basswood family and is very nutritious, and known to have medicinal properties as well. Introducing Egyptian spinach as a specialty crop in Texas requires examining and determining the best cultural practices needed for its successful production. The objective of this study was to determine the effects of three nitrogen fertilizer rates on the growth of Egyptian spinach grown under greenhouse conditions. We hypothesized that the yield of Egyptian spinach will increase with increasing nitrogen fertilizer rate. Seedlings of equal sizes were transplanted six weeks after planting into 15 cm plastic containers with Sunshine Professional Growing Mix. The plants were fertilized weekly with a micronutrient (i.e. boron, copper, iron, manganese, molybdenum and zinc) containing fertilizer, All Purpose MiracleGro fertilizer [24-8-16], (Scotts Miracle-Gro Products Inc., Marysville, OH) at 94, 188, and 376 kg N ha<sup>-1</sup> for about 4.5 months. Weekly harvesting of the fresh shoots and weighing commenced nine weeks after transplanting and continued for approximately nine additional weeks. At the conclusion of the study, the results showed that the 94 kg N ha<sup>-1</sup> rate of MiracleGro provided a significantly higher shoot biomass yield than the other treatments. The results imply that fertilizing Egyptian spinach at the 94 kg N ha<sup>-1</sup> fertilizer rate is the best way to optimize yields under similar growing conditions. The results also suggest that optimizing field production of Egyptian spinach may require supplementing soils with micronutrients.

## Introduction

Egyptian spinach (*Corchorus olitorius*, L.) also called Jews mallow or jute mallow, is a popular vegetable that grows in the wild and is also widely cultivated in parts of Africa (Oboh et al., 2009; Musa et al., 2010), Asia (Oboh et al., 2009) and the Middle East (Islam, 2013). It is an erect annual herb that can grow up to 150 cm.

The leaf, which is the edible part, is mucilaginous like okra. Egyptian spinach is nutrient-rich, containing iron, calcium, thiamin, riboflavin, niacin, folate, protein, and dietary fiber. It also contains magnesium, vitamin C, E and  $\beta$ -carotene, galactose, galacturonic acid, glucose, glucuronic acid, and rhamnose (Fondio and Grubben, 2004; Ndiovu and Afolayan, 2008).

Egyptian spinach has several medicinal uses. It is a diuretic, demulcent, and can improve bowel movements (Ogunrinde and Fasinmirin, 2011; Musa et al., 2010). Documentation of its use for treating chronic cystitis, fever, gonorrhoea, and tumor also exists (Oyedele et al., 2006; Zakaria et al., 2006). Ionone glucoside extraction from the leaf may inhibit the activities of histamines (Grubben, 2004). In addition to its nutritional and medicinal attributes, it produces jute fiber.

Research has shown that introducing new crops can provide food options for the growing population and increase farmer income. Notwithstanding, the successful introduction of a crop is dependent on adaptation to local production conditions (Prohens et al., 2003). Hence the need for research to determine best production practices includes identification and optimization of nutrient requirements under local conditions. There is a market for Egyptian spinach in Texas and beyond. Consumers eat both the fresh and dry forms of the leaves of Egyptian spinach; hence there is an opportunity to reduce post-harvest losses by drying the leaves. The objective of this study is to determine the effect of three fertilizer rates on the growth of Egyptian spinach grown under greenhouse conditions. The hypothesis is that the yield of Egyptian spinach will

increase with increasing nitrogen fertilizer rate. The knowledge gained from this study will facilitate field trials of Egyptian spinach, especially cultivation in containers and raised beds in urban areas.

## Materials and Methods

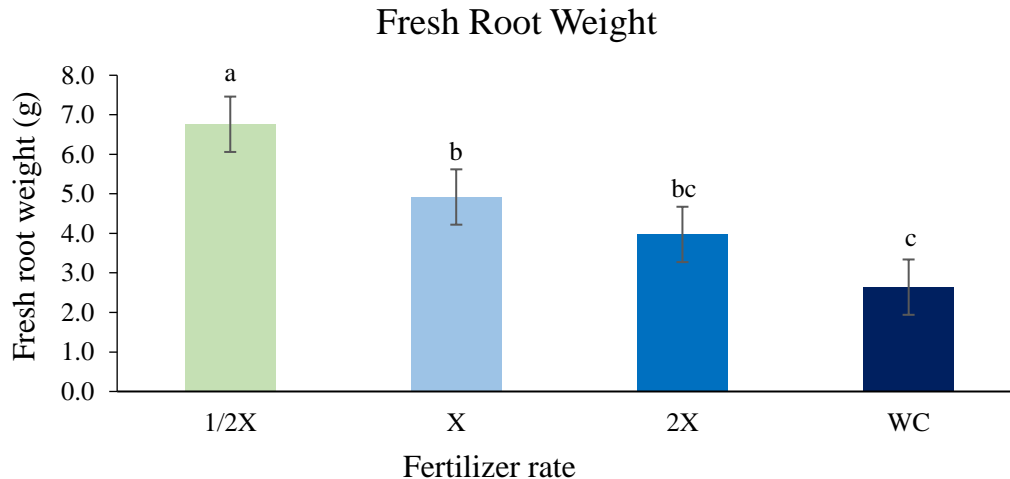
This study was carried out in a greenhouse at the school farm of Prairie View A&M University. Egyptian Spinach (a West African variety) was planted in a flat tray in June 2015 and raised into seedlings for transplanting. Six weeks after planting, seedlings of equal size were selected and transplanted into pots (15 cm wide) containing Sunshine Professional Growing Mix (Sungro Horticulture, Agawam, MA). The seedlings were treated with All Purpose MiracleGro (24-8-16) fertilizer (Scotts Miracle-Gro Products Inc., Marysville, OH) at four rates: 94 kg N ha<sup>-1</sup> (half the label rate), 188 kg N ha<sup>-1</sup> (label rate or the recommended rate), 376 kg N ha<sup>-1</sup> (twice the label rate) and water control/no fertilizer treatment. The label rate for All Purpose MiracleGro (24-8-16) fertilizer, which is 188 kg N ha<sup>-1</sup>, is equivalent to one tablespoon of 24-8-16 per 3.785 L (i.e. 1 gallon) of water. The fertilizer treatments were arranged in a completely randomized design. Each treatment had five replicates. As a result, the transplants assigned to the various fertilizer treatments were arranged accordingly on the same greenhouse bench. The fertilizer was applied weekly for about 4.5 months. An equal volume (250 ml) of the treatment solutions were applied to their respective pots on a weekly basis. Watering of Egyptian Spinach was done by applying the same volume (250 ml) of water to all treatments when needed. The same growing conditions were maintained in the greenhouse for all the treatments during the entire growing season.

Harvesting was done on a weekly basis after two months (starting in late August 2015) by cutting the apical tender parts of the plants including leaves (referred to as fresh shoot or biomass in this paper). Biomass harvested was weighed to obtain fresh weight. At the end of the study, each remaining plant was harvested and separated into roots and shoots. The shoots were weighed to obtain shoot biomass yield. The roots were washed, dried with a paper towel, and weighed as well. An analysis of variance (ANOVA) was completed using JMP software (SAS Institute, NC). Treatment effects were considered significant at  $P \leq 0.05$ .

## Results

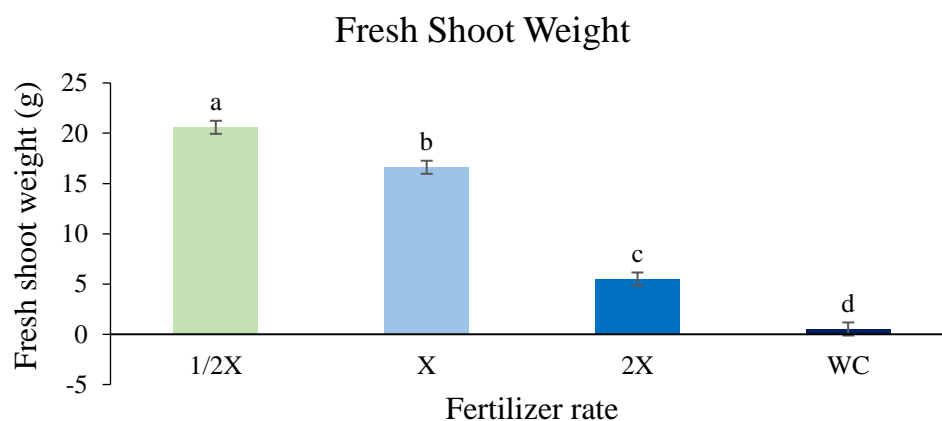
### Treatment effect on fresh root weight and shoot yield of Egyptian spinach

Fresh root weight for the 94 kg N ha<sup>-1</sup> (1/2X) rate was significantly different from the other treatments. The 188 kg N ha<sup>-1</sup> (X) and 376 N ha<sup>-1</sup> (2X) fertilizer rate treatments were statistically the same, but the 188 kg N ha<sup>-1</sup> rate was different from the control (WC) [Fig.1]. Average fresh root weight for the 94 kg N ha<sup>-1</sup> rate was 27%, 41% and 61% greater than 188 kgN ha<sup>-1</sup>, 376 kg N ha<sup>-1</sup> rate and control (WC) respectively. Fresh root weight of the 188 kg N ha<sup>-1</sup> (X) rate was 46% greater than the control.



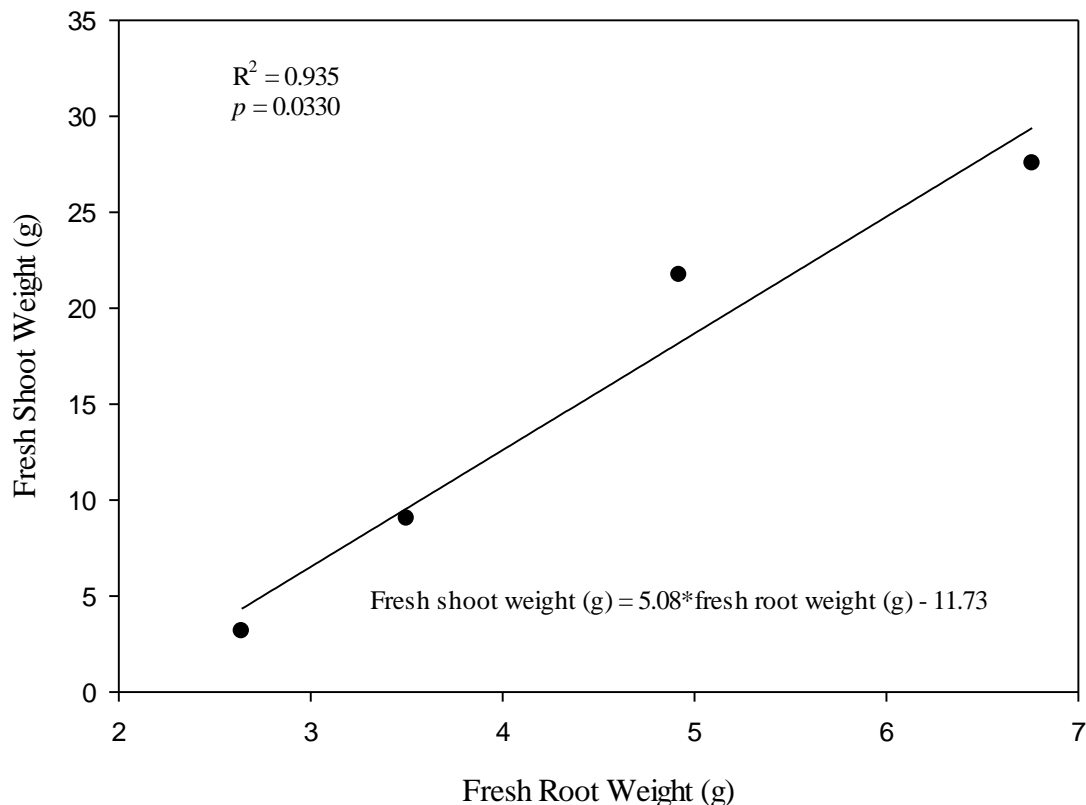
**Figure 1.** Effect of fertilizer rate on fresh root weight of Egyptian Spinach. Each bar represents an average of five treatment replicates while the error bars represent standard errors of the mean. 1/2X = half the label rate (94 kg N ha<sup>-1</sup>), X = label rate of fertilizer (188 kg N ha<sup>-1</sup>), 2X = twice the label rate (376 kg N ha<sup>-1</sup>), WC = water control or no fertilizer treatment. Bars with the same letter(s) are not significantly different from each other ( $P > 0.05$ ).

The results for fresh shoot biomass followed a similar trend as fresh root weights. However, in this case, fresh shoot weights for all the treatments were significantly different from each other (Fig. 2). The 88 kg N ha<sup>-1</sup> (1/2X) rate yielded 19%, 73% and 97% more shoot biomass than 188 kg N ha<sup>-1</sup> (X) and 376 N ha<sup>-1</sup> (2X) and control treatments respectively. Similarly, the average shoot biomass produced by the 188 kg N ha<sup>-1</sup> (X) was 67% and 97% higher than the 376 N ha<sup>-1</sup> (2X) and control treatments respectively. Compared to the control, the 376 N ha<sup>-1</sup> (2X) rate produced 90% more shoot biomass.



**Figure 2.** Effect of fertilizer rate on fresh shoot weight of Egyptian Spinach. Each bar represents an average of five treatment replicates while the error bars represent standard errors of the mean. 1/2X = half the label rate (94 kg N ha<sup>-1</sup>), X = label rate of fertilizer (188 kg N ha<sup>-1</sup>), 2X = twice the label rate (376 kg N ha<sup>-1</sup>), WC = water control or no fertilizer treatment. Bars with the same letter(s) are not significantly different from each other ( $P > 0.05$ ).

Fresh shoot biomass weights for the 94 and 188 kg N ha<sup>-1</sup> rates were more than 3-fold greater than 376 kg N ha<sup>-1</sup> rate and the control (Fig. 2). A significant correlation ( $p = 0.0330$ ) was observed between root biomass and fresh shoot yield (Fig.3).



**Figure 3.** Correlation between fresh root weight and fresh shoot weight of Egyptian spinach grown at three nitrogen (N ha<sup>-1</sup>) rates.

## Discussion

Egyptian spinach treated with 94 kg N ha<sup>-1</sup> rate produced the highest shoot biomass yield compared to the other treatments investigated (Fig.2). As a result, the hypothesis that the yield of Egyptian spinach will increase with increasing nitrogen fertilizer rate was rejected. The practical significance is that farmers can save money on fertilizer and, at the same time, get a better yield when the 94 kg N ha<sup>-1</sup> rate is applied. The results also suggest that bigger plant roots can help improve yield probably because they help draw more water and nutrients for plant use, thereby improving nutrient use efficiency. The strong correlation between fresh root weight and fresh shoot weight (Fig.3) suggest that shoot biomass yield of Egyptian spinach could be predicted using root data for plants grown under similar conditions. Since the MiracleGro fertilizer used contained micronutrients, in addition to the macronutrients (nitrogen, phosphorus and potassium), it is likely the suite of micronutrients including boron, copper, iron, manganese, molybdenum and zinc in the fertilizer may have collectively influenced the results obtained from the study.

## Conclusions

The yield of Egyptian spinach varied with fertilizer rate in this study, and root biomass was strongly related to shoot yield. Nitrogen applied at a 94 kg ha<sup>-1</sup> rate produced the most yield and root biomass. Higher and more concentrated nitrogen rates reduced yield, suggesting Egyptian Spinach does not need a lot of nitrogen for growth and productivity. MiracleGro contains micronutrients; therefore, optimizing yield may involve adding these nutrients to fertilizer programs. The potential savings on fertilizer could make the productions of Egyptian spinach more economical and beneficial to small scale producers.

## Acknowledgments

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**Exploring the Association Between Nutrition and Mental Health in Adolescence: A  
Systematic Literature Review**

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## Abstract

**Introduction:** Throughout life, proper nutrition is important to the brain as it affects cognition and intellectual development. Studies have shown that a lack of certain nutrients can affect the body's ability to perform mentally and physically. Annually, 14% of children in the United States receive mental health illness diagnoses. However, the link between nutrition and mental health is not clear. The purpose of this systematic literature review on mental health and nutrition in adolescence is to identify any gaps that require future research efforts. **Methods:** The Elton Bryson Stephens Company (EBSCO), ProQuest, and Journal Storage (JSTOR) databases were used to search the following terms: nutrition, diet, and mental health. All search results that were published in English between 2014 -2019, conducted in the United States, peer-reviewed, and contained subjects 13 to 18-years old were included. **Results:** A total of 217 articles were identified. After the removal of duplicates and eligibility screening, only two satisfied the criteria. The reports were published in two separate journals during 2018 (n=1) and 2014 (n=1). These quantitative studies used a cross-sectional design with a survey. Common findings across the two studies are (1) nutrition knowledge and frequent family meals are positively associated with the individual social and emotional wellbeing; and (2) poor nutrition can lead to increased bullying among adolescents. **Conclusion:** The findings from this study suggest that nutrition and mental health in adolescents is not well research. Future research studies are needed to address mental health and nutrition among adolescents.

**Keywords:** Nutrition, Diet, Mental Health, Adolescence, Systematic Literature Review

## Introduction

Mental health disorders are complex conditions that affect approximately 50 percent of American adolescents (National Association of Mental Illness, 2019). These disorders include depression, anxiety, stress, and emotional, psychological, and social impairment. According to the National Association of Mental Illness (NAMI), about 7.7 million children between 6 and 18-years old experience a form of mental illness throughout their lifetime, and 22 percent have severe impairments (2016). Several studies sought to understand possible causes of mental health disorders and identify interventions that may eliminate symptoms (Friis, Johnson, Cutfield, & Consedine, 2016; Maalouf & Brent, 2012; Moritz et al., 2016). For instance, investigators have examined how genes mechanisms shape the neuroendocrine response to stress (Ouellet-Morin et al., 2013). Other researchers have explored the possible effects of diabetes on mood change (Friis et al., 2016; Otto et al., 2018; Wherrett et al., 2018), and implications of socio-demographic characteristics on individual behavior (Gary, Stark, & LaVeist, 2007). Studies have shown that inadequate consumption of certain nutrients such as calcium, B-vitamins, minerals and protein can affect the body's ability to perform mentally and physically (Carroll, Samek, & Zepeda, 2018; Leech, Worsley, Timperio, & McNaughton, 2018). However, few researchers have studied the association between mental health and nutrition (Gary et al., 2007; Khosravi et al., 2015).

Adolescence is a period during the developmental stages when children experience drastic hormonal, behavioral, sexual, physiological, and neurological changes. Adolescents need adequate nutrition to achieve their full growth potential and decrease the odds of impaired organ development. For example, amino acids are essential for muscle growth, calcium and vitamin D accommodate bone growth, and some minerals are needed to facilitate nerve functioning (Kolb, Mychasiuk, & Gibb, 2014). One study has documented that childhood malnutrition and a deficiency in micro- and macronutrients can lead to chronic disease during adulthood (Sánchez-Villegas et al., 2015). Another clinical study has documented that supplementation with zinc and omega-3 fatty acid can reduce symptoms of depression and hyperactivity disorders (Gowda, Mutowo, Smith, Wluka, & Renzaho, 2015).

Research on mental health and its association with the environment, personal experiences, and genetic make-up (DNA) are well explored. However, there are not many conversations about how the type and quality of foods consumed may affect mental health. Therefore, the purpose of this systematic literature review is to identify research gaps that exist that may shed light on the relationship between mental health and nutrition in adolescence.

## Methods

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009) and was conducted to summarize published empirical studies that focused on mental health and nutrition among adolescents. In September 2019, three databases Elton Bryson Stephens Company (EBSCO, ProQuest, and Journal Storage (JSTOR) were searched for articles relating to mental health and nutrition. Search terms include “nutrition,” “diet,” and “mental health.” The search terms were chosen based on the researchers’ expertise and a cursory review of the literature. Publication years were limited to 2014 through 2019 because new recommendations are published every ten years. Articles were included if they were published in English between 2014 - 2019, peer-reviewed, conducted in the United States, and studied adolescents 13 to 18-years old. All review articles and videos were excluded along with any article not published in the United States.

## Data Extraction

Two investigators collected the following data from all studies: first author name, publication date, title, research aims or purpose, study design and setting, research methods, and classification of mental health and nutrition. Two screening procedures were conducted on all retrieved articles. The first screening procedure was done on abstracts to ensure the articles met the established criteria. Articles that did not meet the established criteria were excluded. For the second screening, the eligible articles were read in their entirety. At this phase, some articles were excluded because they mentioned mental health and nutrition in adolescence in the title, but the content was about the impact of maternal nutrition during pregnancy on adolescence.

## Assessment of Mental Health and Nutrition Components

An open-ended questionnaire was developed to assess components of mental health and nutrition. This tool allows for a range of responses (Table 1).

**Table 1:** Criteria for Assessing the Studies on Mental Health and Nutrition

Components	Questions sample	Options
Mental Health	Which aspect of mental health included in this study?	Depression, anxiety, stress
Nutrition	Which aspect of nutrition is included in this study?	Food consumption, nutrients

## Quality Appraisal and Methodological Quality Assessments

To ensure that coding and extraction were consistent across all articles, we first developed a coding instrument in excel. After a critical examination of abstracts, all non-relevant studies were eliminated, and the remaining full articles were reviewed. The quantitative methodological quality scale was adopted from a prior study (Lu et al., 2014), with minor changes (See Table 2).

**Table 2:** Critical Assessment of the Quantitative Studies n= (2)

Methodology	Description	Score	# of studies
<b>Study design</b>	Experimental study (e.g., randomized control trial)	4	0
	Case-control study	3	0
	Longitudinal study	2	1
	Cross-sectional study	1	1
	Did not indicate	0	0
<b>Sample size</b>	Large (>300)	3	1
	Medium (>100 and <300)	2	0
	Small (<100)	1	1
<b>Data analysis</b>	More advanced statistics (e.g., mixed models)	4	0
	Regression/analysis of covariance, Bivariate statistics (e.g., ANOVA, Pearson r, t-test)	3	1
	Descriptive only (e.g., frequency)	1	1
<b>Control variable(s)</b>	Included	1	1
	Not included	0	1
<b>Data reliability testing</b>	Reported results	1	0
	Not reported	0	2
<b>Data validity testing</b>	Reported results	1	0
	Not reported	0	2

## Results

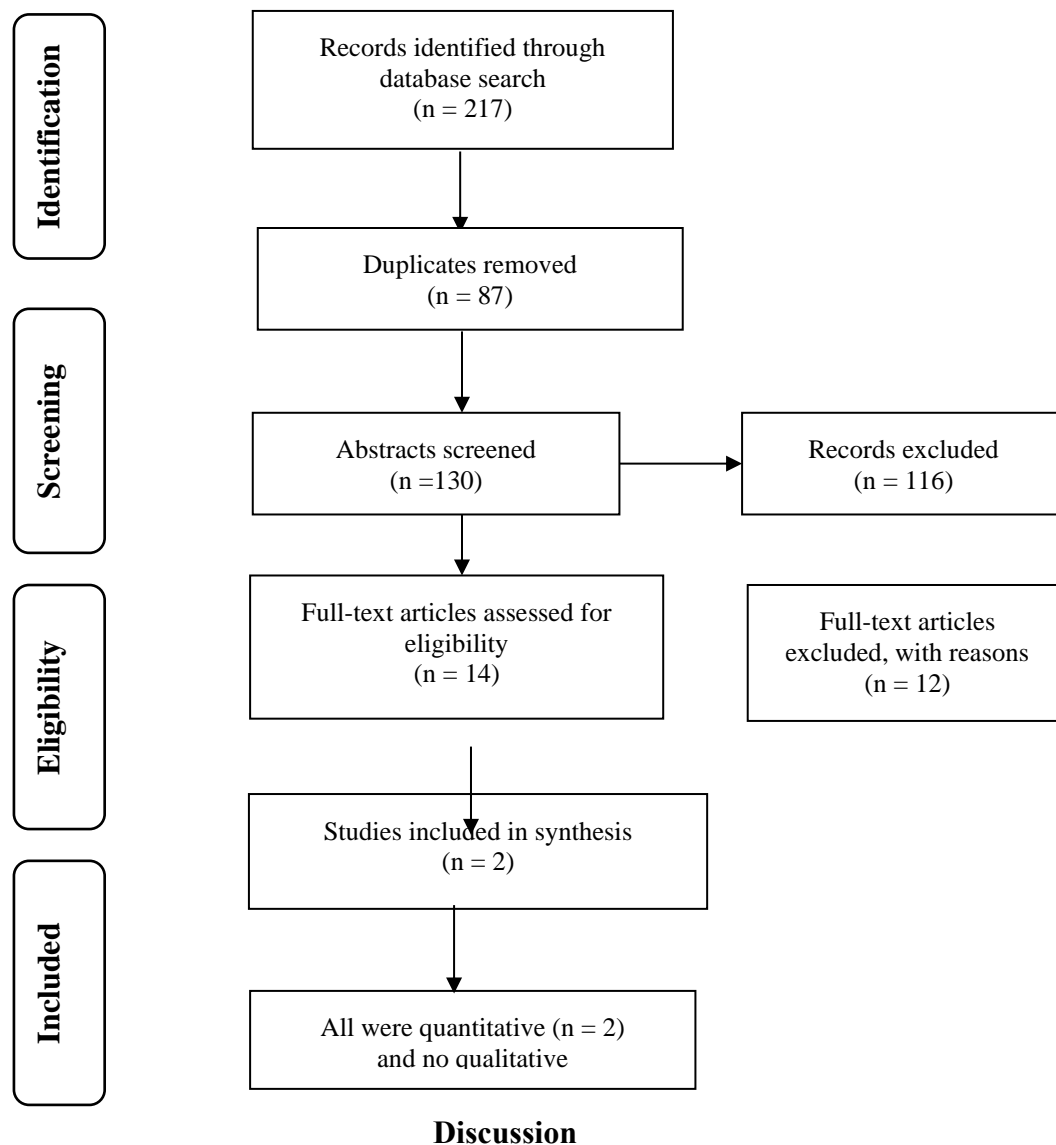
A total of 217 articles were identified during our first search (Figure 1). A significant portion (87, 40%) were duplications and 130 articles underwent abstract screening. After we assessed for our inclusion criteria, 116 were excluded because the articles provided nutrition information on assessment and intervention on children below our target population age. Only fourteen (n=14) articles met full criteria. Eight of these contain supplementary information about nutrition and mental health and four were review articles. For the two articles that met inclusion criteria, we extracted authors, settings, published years, study purpose, study settings, and design and selected findings, as shown in Table 3.

**Table 3:** Characteristics of Nutrition and Mental Health studies (n=2)

Lead Author/ Year/Journal Name	Study Purpose	Subjects/ Sample Size	Study Methods & Settings	Component of Mental Health	Com- ponents of Nutri- tion	Selected Findings
Judith O'Hare, (2014); <i>Journal of Pediatric Health Care</i>	To determine the relationships among body mass index, healthy lifestyle beliefs and behaviors, and mental health indicators for 5th and 6 <sup>th</sup> -grade children in a Title I school	Male & Female N=45	Quantitative, School	Depression, Anxiety, and Self-concept	Whole Milk, Salty Foods	Knowledge about nutrition was not significantly correlated to any of the outcome measures in this population except activity knowledge ( $r=0.459$ , $p<.01$ ). Higher activity knowledge was positively correlated to healthy behaviors ( $r=0.375$ , $p<.05$ ) and significantly correlated to the child's belief that they could live a healthy lifestyle ( $r=0.410$ , $p<.01$ ).
Rachele Pojednic (2016); <i>Contemporary Clinical Trials Review</i>	To compare behavioral health risk and protective factors (e.g. nutrition, emotional and relationship scales, vitality and energy, student engagement, stress, positive affect, self-efficacy, and life satisfaction) and academic performance between Build our Kids Success (BOKS) participants and control students.	Elementary and Middle School Students N=1490	Quantitative, School	Experienced emotions related to anger, anxiety, sadness, fatigue, and interactions with peers.	Fruits and vegetable con- sumption	Applying a within-school controlled study design allows for direct comparison within each individual school as well as control for between-school variation.

One article was published in the *Pediatric Health Care Journal* (O'Haver, Jacobson, Kelly, & Melnyk, 2014) and the other was published in *Contemporary Clinical Review* (Pojednic, 2016). Both peer-reviewed articles were quantitative studies and used a cross-sectional design with a survey. The longitudinal study design was used by Pojednic (2016) who evaluated a program for mental health and nutrition. None of the articles reported reliability. Haver and colleagues studied eating behavior by analyzing frequency of fruit, vegetable, whole milk, and salty food consumption (2014). Pojednic and colleagues explored food consumption and associations with individual social and emotional wellbeing (2016). Both studies looked at anxiety and depression as it relates to food. Pojednic (2016) examined anger, sadness, and fatigue with eating behavior, while Haver (2014) explored self-concept and junk foods.

*Figure 1. Systematic Literature Search for Articles Published Between 2014 and 2019 Related to Mental Health and Nutrition in adolescents. Note. This flowchart is an adaption of Garrard, Judith (2013).*



This systematic literature review seeks to assess the current literature for research conducted on mental health and nutrition in adolescents between 2014 and 2019 and identify any gap that may influence future research. Our study retrieved articles from EBSCO, ProQuest, and Jstor. Oniel and colleagues published a systematic literature review (SLR) in 2014 that examined the relationship between diet and mental health in children and adolescents (O'Neil et al., 2014). The researchers recommended using a longitudinal design to understand the specific biological mechanism of action as it relates to mental health (O'Neil et al., 2014).

The current study found that limited studies are being conducted on mental health and nutrition in the United States among adolescents. The most interesting finding was that no articles were published in 2015, 2017, 2018, and 2019. Since the National Association of Mental Illness (NAMI) reported almost fifty percent of adolescents experience mental illness, and food consumption has been shown to have some implication on health, there is a need to understand if there is any link between the two. A possible explanation for these results may be the lack of adequate information and research in this area. Future research must address food quality and possible outcomes as it relates to mental health.

Our methodological quality assessment revealed one study included longitudinal design. However, none of the studies reported the reliability and validity of the instruments used. Also, none of the studies used qualitative or mixed-method approach or randomization. While cross-sectional design and convenience sampling are widely used in the literature, randomized and experimental designs are the most rigorous processes in research. Therefore, future research may need to emphasize randomizing participants and validating the instrument for reliability and validity.

Although the study has successfully demonstrated needs for more research on mental and nutrition among adolescents, it has certain limitations in terms of literature search. The articles used in this study were retrieved through the Prairie View A&M library. Therefore, the search strategy may not capture all the relevant articles. Our review only employed three databases, so some relevant journals may not index such as PubMed. Also, the search terminology may not have been broad enough to capture all published research on mental and nutrition among adolescents.

## **Conclusion**

The main goal of this study was to identify published empirical articles and determine the gaps in the current literature. The findings from this study suggest that nutrition and mental health in adolescents is not well research. Future research studies are needed in mental health and nutrition among adolescents.

## **Acknowledgments**

We would like to thank the Prairie View A&M Library for allowing us to use their computers to retrieve these articles. Thanks to Miss Kimberly Gay and Dr. Lenna Dawkins-Moultin for their support.

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## **Biological Pathways Associated with Wild and Domestic Animals**

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## Abstract

**Background:** Zoonotic diseases are problematic, in that, they impact both wild and domestic animals alike. Thus, there is a need to investigate the genomes of wild and domestic. Gene ontology (GO) is a major bioinformatics initiative, whereby descriptions of gene products across the database are developed and unified to describe all species. This process is performed by biocurators, who gather, annotate, and validate information on the databases, consequently affording easy access to accurate and updated data. In this study, we investigated the biocuration of two biological processes, DNA integration, which is used for DNA coding, and the tricarboxylic acid (TCA) cycle that occurs in all aerobic organisms. The objective of this study was to compare the genomes of 271 mammals, birds, reptiles and some aquatic animals to determine the number of wild versus domesticated animals, where DNA integration and the TCA cycle have annotations. We hypothesized that there would be more annotations on domesticated animals than wild animals because of easier access to domesticated animal genomes. **Methods:** To test this hypothesis, we first accessed the National Center of Biotechnology Information (NCBI) to retrieve the taxonomy ID for 271 species of animals found in this study. Then the European Bioinformatics Institute (EBI) database, QuickGO, was accessed to retrieve all annotations associated with the taxonomy ID of the species. Data was assembled into a wiki-database that is now publicly available online. **Results and Conclusion:** Data indicated that more annotations for DNA integration and the TCA cycle were 22% higher in domestic animals than in wild animals. Therefore, we propose that more biocuration needs to be done for wild animals. The number of wild animals' sequences available are growing, but if they are not annotated, detailed investigations are not possible. If more resources are dedicated to the investigation of the genomes of wild animals, more work can be done to study the genetic factors affecting zoonotic diseases.

**Keywords:** bioinformatics, zoonotic diseases, wildlife, domesticated animals

## Introduction

Information is constantly being acquired in the life sciences, and with an abundance of informational databases, it is a necessity to keep collected works up to date and accessible to the rest of the world. Genomic databases are used by many people, including students and researchers (Hayamizu, 2015). Furthermore, databases have become an essential part of the biological sciences, because they often serve as gateways to biological data (Binns, 2009). Biocurators take information from scientific literature and describe the data using annotation protocols (Jenkinson, 2008). Additionally, biocurators assign Gene Ontology terms (GO terms) to a specific gene product, referred to as GO annotation. These GO annotations represent a biological process, molecular function or cellular components (Blake, 2014). The European Bioinformatics Institute (EBI) database collects and stores data from life science experiments and provides many services and tools such as Quick Gene Ontologies (QuickGO) (Squizzato, 2015). Quick GO is a GO annotation database that allows scientists to share data, perform complex queries and analyze results (Ashburner, 2000).

There are several annotations that could be used to compare the genomes of wild and domesticated animals. For this experiment, the annotations for the Tricarboxylic Acid (TCA) cycle and DNA integration were chosen and the rationale for this choice follows. Both the TCA

cycle and DNA integration processes are known to be functional in animals. Although these two processes are individually being studied by scientists, the number of annotations for these processes in domestic and wild animals is unknown. Because many species are the subject of current genomic investigations, the sequencing data of wild and domestic animals are now publicly available and will allow for a comparison.

The TCA cycle is a very important biological process that produces energy and is known to occur in all animals (Alisdair, 2004). The energy production cycle is also found in most plants, animals, fungi, and bacteria. In this experiment, we chose the TCA cycle as our control, as it is found in almost all genomes. Thus, the number of TCA cycle annotations should be consistent regardless of whether the species is wild or domesticated.

DNA integration is the biological process that controls the integration of foreign material into the DNA of a host cell. Mammalian genomes are susceptible to foreign DNA insertions both naturally and experimentally. Meaning that viruses that are DNA- or RNA-based viruses can integrate into the mammalian genomes with ease (Müller, 2001). Mechanisms involved in the illegitimate integration of DNA could also be involved in viral DNA integration (Würtele, 2003). Viral DNA integration and retroviral DNA integration share similar characteristics as DNA integration in mammalian cells, but they are only found within prokaryote cells. DNA integration is represented in the host cell of the mammalian genomes. Therefore, we chose to use DNA integration annotations, as these annotations mark areas of a genome that have been changed or impacted by a virus within the host cells. We want to isolate this biological process for its annotations within the wild and domesticated animal genomes, but since DNA integration is not as prominent as TCA cycle, there might not be a consistency of annotations.

The objective of this study is to compare the genomes of 271 variety of mammals, birds, reptiles and aquatic animals to determine the number of wild versus domesticated animals, in which DNA integration and the TCA cycle are annotated. Since the TCA cycle and DNA integration occurs in all organisms and are both important biological processes for the organisms studied, we hypothesized that there would be more annotations on domesticated animals than wild animals, because of easier access to domesticated animals for genetic investigations.

## Methods

The methods used involved utilizing three main databases. National Center of Biotechnology Information (NCBI), European Bioinformatics Institute (EBI) and a wiki-database. The wiki-database was created to house the information collected in this study. For this experiment, we focused on mammals, reptiles, fish, and birds. All data was transferred into the wiki-database on the “Species Table Page.” (Figure 1). A separate table was created for each animal that was investigated. Each species table shows the following genomic information: the common name, category, genomic status (genome sequencing), the research lab (in which the genome was sequenced), link sources among other (Figure 1).

The wiki-database houses all the information collected on animals, plants and insects. We separated the species into different categories that determined the importance of those species. The wiki-database housed the genomic information needed to create a summary of each “Species Table” for the 271 species investigated.

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### Objectives [edit]

1. Find species that are agriculturally important.
2. Assess GO annotations
3. Pick biological pathways.
4. Provide a survey of functional annotation for selected pathways for agriculturally important species of your interest.

### List of Agriculturally Important Species [edit]

Some possibly useful resources:

- [USDA Agricultural Research Service](#)

### Species Table

Category	Species Name	Common Name	NCBI Taxon ID	Genomic Status	Research Lab(s)	Link(s)	Additional Notes
Insect (Pest)	<a href="#">Icerya purchasi</a>	Cottony Cushion Scale	<a href="#">249532</a>	No Data		<ul style="list-style-type: none"> <li>• <a href="#">Wikipedia</a></li> <li>• <a href="#">Wikipedia</a></li> <li>• <a href="#">Texas A&amp;M Agrilife Extension</a></li> <li>• <a href="#">Biocontrol University of California Riverside</a></li> <li>• <a href="#">Journal of Economic Entomology</a></li> <li>• <a href="#">International Organization for Biological Control</a></li> </ul>	Icerya Purchasi is a pest to citrus and pittosporum.

Figure 1: Wiki-database for information collected on the species investigated.

To use NCBI, we first had to find the binomial nomenclature for each animal using their common names. Next, we identified whether the species was wild or domesticated by using information stored in the NCBI Taxonomy database. A new table was created for each of the 271 species investigated

We assessed Gene Ontology (GO) annotations in the EBI, QuickGO database for each species. Lastly, we inserted GO annotation data found into our “GO Annotation Survey”. The “GO Annotation Survey” is linked to each species in the “Species Table” under their species name. The “GO Annotation Survey” shows display the NCBI taxonomy ID at the top. Then goes into further detail of the breakdown of different categories that are shown in the far-left column in the survey. The middle column houses the proteins/annotations found for that taxonomy ID, then the next two columns display the individual biological pathways and their proteins/annotations found.

## Results

The graphs in Figures 2 & 3 break down the biological pathways for species with annotations versus the total number of species investigated. Below the graph shows the overall comparison of the domesticated species and the wild species and how they vary within the evidence found.

Of the 71 domesticated mammals, fish and reptilian species examined only 1% (1/71) had DNA integration markers annotated, 8% (6/71) had the TCA cycle and 28% (20/71) had both DNA integration and TCA cycle markers annotated in their genomes. The number of annotations found for each biological pathway was not in abundance. Yet, enough to prove that there are annotations for the domesticated species that were found in this study.

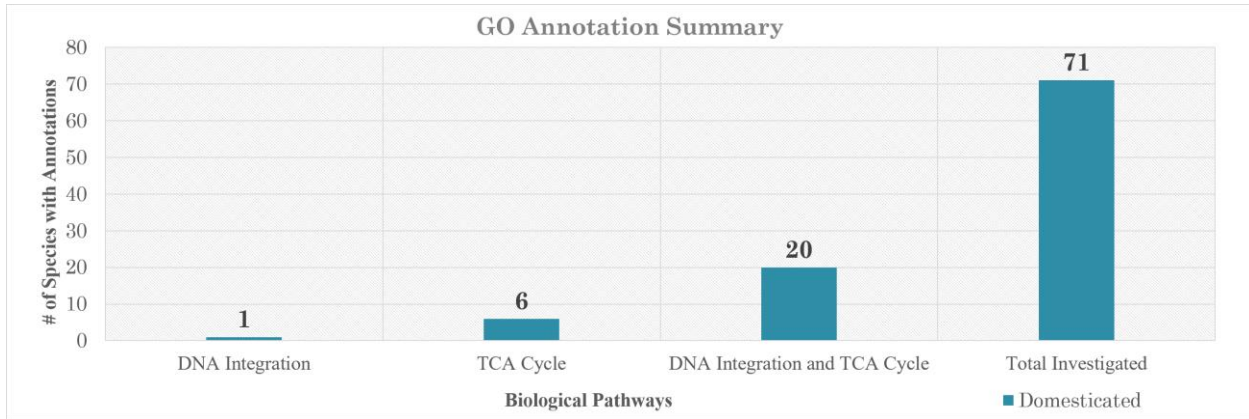


Figure 2: GO Annotation Summary

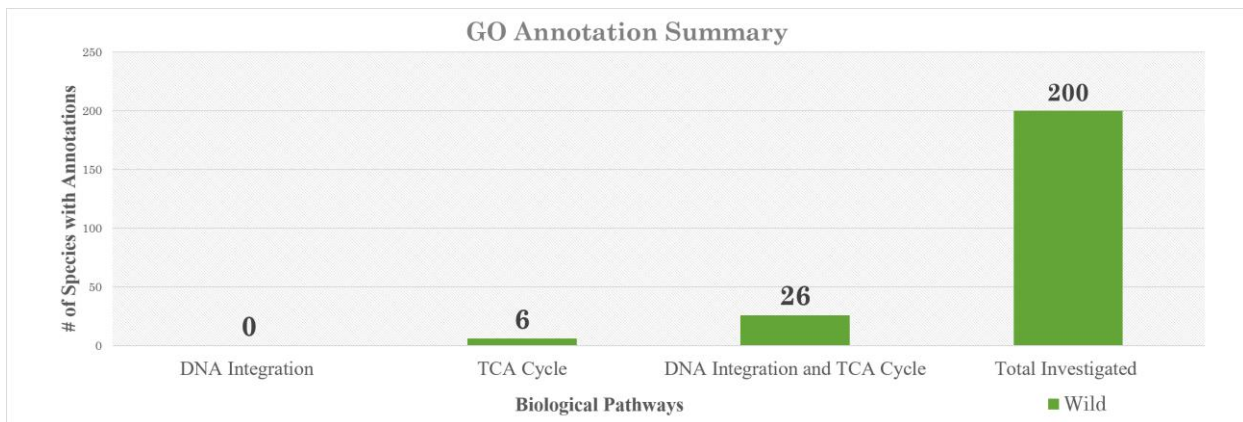


Figure 3: Wild Species Go Annotation Summary

Of the 200 wild mammal, fish, and reptilian species investigated, 0% (0/200) had DNA integration alone, 3% (6/200) had TCA cycle and 13% (26/200) had both DNA integration and TCA cycle. The number of annotations found for each biological pathway was not as expected but results indicate that some wild species did have annotations associated with their species.

### Overview of GO Annotation Survey

After identifying the genomes with annotations using the GO Annotation surveys. We formulated Wiki species pages. Table 1 shows the GO Annotation survey for *C. hircus*. It shows the important components addressed in the methods.

As shown in Table 1 below, you will see that it displays the NCBI Taxonomy ID at the top. The left column below the Tax ID shows all the proteins and GO Annotations for the species. The right side shows the GO term and ID for the TCA cycle and DNA integration. The Parent GO ID and term name are for the biological processes investigated. The survey also shows the total number of GO Annotations which are broken down into the evidence and the

Table 1: Survey of *Goat Species* Wiki Page and GO Annotation

NCBI Taxonomy ID	9925		
Parent GO ID	GO:0006099	GO:0015074	
Parent GO Term Name	TCA	DNA Integration	
Number of Proteins	22,479	2	0
Total Number of GO Annotations	21,565	2	0
Electronic Evidence	20,850	2	0
Manual Evidence	21	0	0
Molecular Function Aspect	6,555	0	0
Biological Process Aspect	8,080	2	0
Cellular Component Aspect	7,844	0	0

aspects. For each species, they have annotations that display electronic and manual evidence and then they are categories in either molecular function, biological processes, or cellular components.

There are two main types of evidence used to separate the annotations, electronic and manual. Electronic evidence is a simple algorithm that biocurator uses to search for the desired gene products such as the TCA cycle pathway. These are automatic annotations imputed by biocurators, but they are essentially hypothetical annotations. This causes the total number of GO annotations to fluctuate because electronic evidence will be deleted or added at any time. Thus, the importance of the need for manual evidence as well. In QuickGO, there are multiple different types of manual evidence used but we chose to look for manual evidence that is found from experimentation. This type of manual evidence is important because it provides accurate annotations for each species that we investigated.

There are three different aspects used to categorize each annotation. *The molecular function* is how the gene product performs, the *biological process* is that gene products involved in, and which *cellular components* the gene products are found. We input data at each aspect for the total 271 species. Both the TCA cycle and DNA integration both fall into the aspect of the *biological process aspect*.

We compare the differences between both the wild and domesticated species to test our hypothesis that there would be more annotations on domesticated animals than wild animals. Shown in Tables 2 through 4, the domesticated species have a higher amount of annotations when compared to our wild species in Tables 5 through 8. The *B. taurus* (cattle) gene products have many protein products and some annotations for the biological processes. The *O. cuniculus* (rabbit) and the *F. catus* (cat) being that they are both domesticated species are like the *B. taurus* in terms of their GO Annotation surveys. Shown in Tables 5 and 6, we have two of the wild species, *P. Alecto* (black flying fox) and *P. troglodytes* (chimpanzee) used for research that possess a high amount of annotations, yet shown in Tables 7 and 8, the other wild species, *S. tatarica* (Saiga antelope) and *P. lotor* (raccoon), a low amount of gene products are found along with any for the *biological processes* of the TCA cycle and DNA integration.

Table 2: Survey of *Cattle* Species Wiki Page and GO Annotation

NCBI Taxonomy ID	9913		
Parent GO ID	GO:0006099	GO:0015074	
Parent GO Term Name	TCA	DNA Integration	
Number of Proteins	27,886	54	6
Total Number of GO Annotations	353,986	119	7
Electronic Evidence	297,431	107	6
Manual Evidence	3,345	0	0
Molecular Function Aspect	92,295	0	0
Biological Process Aspect	146,821	119	7
Cellular Component Aspect	114,870	0	0

Table 3: Survey of *Rabbit* Species Wiki Page and GO Annotation

NCBI Taxonomy ID	9986		
Parent GO ID	GO:0006099	GO:0015074	
Parent GO Term Name	TCA	DNA Integration	
Number of Proteins	19,673	33	10
Total Number of GO Annotations	223,372	57	10
Electronic Evidence	218,338	57	10
Manual Evidence	739	0	0
Molecular Function Aspect	60,217	0	0
Biological Process Aspect	94,671	57	10
Cellular Component Aspect	68,484	0	0

Table 4: Survey of *Cat* Species Wiki Page and GO Annotation

NCBI Taxonomy ID	9685		
Parent GO ID	GO:0006099	GO:0015074	
Parent GO Term Name	TCA	DNA Integration	
Number of Proteins	25,671	38	12
Total Number of GO Annotations	264,365	76	12
Electronic Evidence	234,771	67	12
Manual Evidence	6	0	0
Molecular Function Aspect	76,197	0	0
Biological Process Aspect	19,542	76	12
Cellular Component Aspect	79,626	0	0



Table 5: Survey of *Black Flying Fox* Species Wiki Page and GO Annotation

NCBI Taxonomy ID	9402		
Parent GO ID	GO:0006099	GO:0015074	
Parent GO Term Name	TCA	DNA Integration	
Number of Proteins	14,382	21	2
Total Number of GO Annotations	78,874	36	2
Electronic Evidence	78,799	36	2
Manual Evidence	0	0	0
Molecular Function Aspect	30,814	0	0
Biological Process Aspect	24,093	36	2
Cellular Component Aspect	23,967	0	0

Table 6: Survey of *Chimpanzee* Species Wiki Page and GO Annotation

NCBI Taxonomy ID	9598		
Parent GO ID	GO:0006099	GO:0015074	
Parent GO Term Name	TCA	DNA Integration	
Number of Proteins	78,081	121	19
Total Number of GO Annotations	533,813	224	19
Electronic Evidence	506,230	216	19
Manual Evidence	5	0	0
Molecular Function Aspect	177,010	0	0
Biological Process Aspect	197,850	224	19
Cellular Component Aspect	158,953	0	0

Table 7: Survey of *Saiga antelope* Species Wiki Page and GO Annotation

NCBI Taxonomy ID	34875		
Parent GO ID	GO:0006099	GO:0015074	
Parent GO Term Name	TCA	DNA Integration	
Number of Proteins	38	0	0
Total Number of GO Annotations	467	0	0
Electronic Evidence	467	0	0
Manual Evidence	0	0	0
Molecular Function Aspect	131	0	0
Biological Process Aspect	143	0	0
Cellular Component Aspect	193	0	0



Table 8: Survey of *Raccoon* Species Wiki Page and GO Annotation

NCBI Taxonomy ID	9654	
Parent GO ID	GO:0006099	GO:0015074
Parent GO Term Name	TCA	DNA Integration
Number of Proteins	266	0
Total Number of GO Annotations	1,913	0
Electronic Evidence	1,913	0
Manual Evidence	0	0
Molecular Function Aspect	440	0
Biological Process Aspect	642	0
Cellular Component Aspect	831	0

When comparing the 71 domesticated species to the 200 wild species, majority of the wild species annotated did not possess a high amount of proteins and GO annotations like the *Saiga antelope* and *raccoon*, leading one to speculate that there is more focus on domesticated species and species widely used for laboratory research. Such works enable the identifications and detail of their genomes annotated.

## Conclusion

During our experiment, we can establish that domesticated and wild species had annotations associated with the biological processes, TCA cycle and DNA integration. We were able to validate the hypothesis because domesticated animals possessed more annotations than wild animals. Though evidence of biological processes was minimal and mostly electronic, it still validates that these two processes found. Additionally, through observation, the findings in this experiment determine that wild species annotated did not possess a high number of gene annotations. Therefore, a lack of GO annotations reflects the poor annotation of genes that are known, or genes being missed in the genome records. The problem of a low quantity of identified genes would suggest is with the structural annotation missing genes, or not finding homologs of the genes present. For example, *S. tatarica*, the lack of DNA sequencing and genome record for this species, justifying the low number of genes. The *P. lotor* also shows there only being a record of the mitochondrial DNA being sequenced, explaining the low count of genes present. We were able to find that more biocuration with wild animals because little is still known about their genomes.

We used bioinformatics to bring awareness of information that is still unknown about most animal genomes. The majority of the domesticated and wild species that were found during this study are vectors for zoonotic and infectious diseases. It is apparent that there is an increasing need for genomics in disease risk and susceptibility for endangered species (Irizarry, 2016). Alonso Aguirre argued that “habitat destruction, globalization, and species loss have led to ecosystem disruptions altering infectious disease transmission patterns” (Aguirre, 2017). That is why we wanted to examine a pathway that deals with foreign DNA transmission. We also wanted to search for gene annotations associated with DNA integration because “some viral DNA integration events are [similar] in nature to any type of foreign DNA integration” (Würtele, 2003). But due to the lack of annotations, we found that more exploration for wild animals for

DNA integration is needed. In using tools like bioinformatics for genomic research, we can make connections that could provide knowledge on zoonotic disease etiology.

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