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Morphometric Response of Peas (*Pisum Sativum*) Using Human Urine-Based Fertilizer and Polychromatic Light Source Under Lunar Regolith Condition

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Abstract
The use of human urine-based fertilizer and artificial light source have garnered interest due to its potential for sustainable agriculture, particularly in challenging environments such as lunar regolith. This study evaluates the efficacy of urine-based fertilizer and polychromatic light source on the growth of pea plants (Pisum sativum) in mixed earth and lunar soil conditions. Utilizing six plastic pots with varying soil compositions, pea plants were cultivated under controlled greenhouse conditions. Human urine diluted in tap water was applied weekly to three experimental pots, while the other three served as controls. Moreover, red and blue light were used with a grow light set on a 12/7 hour on cycle, full spectrum, and 100 percent brightness. Over a six-week period, plant growth parameters, including height, light intensity, pH, and moisture, were measured bi-weekly. The findings revealed that the experimental pots treated with urine-based fertilizer exhibited significantly higher growth rates with 50% earth soil, 50% lunar soil concentration compared to the control pots, indicating that the nutrient-rich urine effectively enhanced plant development, and polychromatic light source with a composition of 40% earth and 60% lunar soil exhibited exponential growth. The study highlights the potential of human urine as a valuable nutrient resource for lunar agriculture promoting both plant growth and sustainable resource utilization. These results suggest that integrating human urine-based fertilizers could address nutrient scarcity and support agricultural efforts in extraterrestrial environments.

Keywords: Human Urine-Based Fertilizer, Morphological Responses, Lunar Regolith Condition Polychromatic Light Source

1. Introduction
Several organic fertilizers have been researched and evaluated for their efficacy and efficiency in encouraging plant development. One of them is human urine-based fertilizer, which shows promising outcomes in terms of plant morphological responses [1]. Discovered that urine has a considerable contribution to soil, resulting in less soil contamination and increased plant development. Additionally, this significance of integration of human urine-based fertilizer is due to the abundance of nitrogen and phosphorous, which are essential components of plant nutrition and development [2]. On that note, using the nutritional ability of human urine-based fertilizer for lunar agriculture not only addresses the issue of nutrient scarcity, but also aligns with the ideals of recycling and resource efficiency. Urine based fertilizer can be a nutrient resource for agriculture.

Moreover, technological integration to natural plant production and development conveys potential approach towards the evolving technological era. Artificial lighting is one of the extensive means on plants growth and quality [3]. Mentioned that light can regulate plant growth, morphology, and metabolism. Moreover, the impact of light to plant development matters on light source associated with the color light spectrum, blue and red light were proved to influence the growth of the plants and increased the normal dynamics and distribution of nutrients through efficient photosynthesis circulation [4]. Light is not only the energy for photosynthesis but also a signal for plant morphogenesis. Light quality and photoperiod have an important impact on plant growth and development, as well as yield and quality.

Pea plants (Pisum sativum) is an important source of high-quality vegetable protein in the human diet. Its protein components are generally considered hypoallergenic, and many studies have highlighted the health benefits associated with the consumption of pea protein [5]. Pea plants are relatively easy to grow and are well suited to home gardens and small-scale agriculture, like using human-urine as a home-based fertilizer [6]. In addition, the human spaceflight realm poses unique challenges for engineers who develop facilities to conduct plant experiments, grow crops, and design biology-based life support systems for off-Earth habitation to support nutrition. Brassica rapa, and Pisum sativum produced viable result. Lacking of light is a major problem in growing plants in space. In the study the growth characteristics of the two pea sprout cultivars were significantly different. LED light treatment had significant effects on plant height and SPAD value of the upper third lea.

This research explores the potential of cultivating pea plants (Pisum sativum) under unique and challenging conditions, specifically utilizing human urine-based fertilizer and varying light intensities in a simulated lunar soil environment. The study aims to address the feasibility of growing crops in extraterrestrial settings by assessing the effects of these factors on the growth and morphological characteristics of peas. By examining the interactions between urine-derived nutrients, light intensity, and lunar regolith properties, this research seeks to determine optimal conditions for plant development during different growth stages and at varying fertilizer concentrations. The findings could contribute to advancing sustainable agricultural practices for long-term space exploration and colonization, offering insights into resource recycling and efficient crop production in non-Earth environments.

2. Hypothesis (Research Question)

How efficient the nutrient absorption from the urine-based fertilizer and light intensity and its influence on the status of the pea plants by assessing the growth and morphological responses of peas (Pisum sativum) under lunar regolith condition? With the presence of diluted human urine-based fertilizer in mixed regolith, the pea's growth increases 2 times faster compare to the common growth duration of the controlled group and wide vascular and ground tissues width and height difference, respectively.

3. Methodology

In this study, we use experimental design. Experimental design is a scientific investigation to do and design experiment which one can analyze the relationship between variables. Generally speaking, one variable, the independent variable is controlled in order to measure its effect on other, dependent, variables (Bell, 2009). In this study, the following procedure as follows:

4. Experimental Procedure

Six plastic pots (15 cm diameter, 13.5 cm height) with drainage holes and saucers; A) two pots of 40% Earth soil and 60% Lunar Soil, B) two pots of 45% Earth soil and 55% Lunar soil and C) two pots of 50% Earth soil and 50% Lunar soil. Three (3) pots for control set up for human urine fertilizer experiment and another three (3) pots for polychromatic light source experiment. For experimental set up six (6) pots in total for human-urine and polychromatic light source study. Pea plant will be planted in each pot, 1.5 cm deep. A mixture of human urine (50ml pure urine in 150ml tap water) used as a fertilizer in applied once a week.

The experiment for human-urine, the study use grows light set on a 12/7 hour on cycle, full spectrum, and 100 percent brightness. Another three (3) pots will be used as control environment with a fluorescent light with a grow light set on a 12/7 hour on cycle, full spectrum, and 100 percent brightness. Moreover, Red and blue light will be used in first three (3) pots in a controlled environmental with a grow light set on a 12/7 hour on cycle, full spectrum, and 100 percent brightness. Another three (3) pots will be used as control environment with a fluorescent light with a grow light set on a 12/7 hour on cycle, full spectrum, and 100 percent brightness.

Pots will be watered daily (on weekdays) from the bottom via their saucers. pH, soil acidity and plant growth (once visible above the regolith/soil), and were measured bi-weekly during success academy every after Saturday where team can meet and do the measurement and writing. The plant is watered daily.

5. Morphometric Measurement

To obtain morphometric measurements for the pea plants in the experiment, the height of each plant should be measured bi-weekly using a ruler or measuring tape, starting from the base of the stem at soil level to the tip of the tallest leaf or stem. The dimensions of a representative leaf, including its length (from the base to the tip) and width (at the broadest point), should also be recorded using a digital caliper or ruler. Additionally, the diameter of the main stem, measured 2 cm above the soil surface with a digital caliper, can provide insights into the plant's structural growth. The number of visible leaves on each plant should be counted bi-weekly to monitor foliage development. At the end of the experiment, plant biomass can be determined by harvesting, separating shoots and roots, and drying them in an oven at 70°C until a constant weight is achieved, followed by precise weighing. Photographs of the plants should be taken bi-weekly under consistent lighting and angles to document visual growth patterns. Environmental variables, including light spectrum (red/blue or fluorescent), soil composition (Earth and Lunar soil ratios), and fertilizer application, should be recorded alongside morphometric data. These measurements can then be logged systematically in a spreadsheet for statistical analysis, facilitating the comparison of growth metrics under different experimental conditions.

6. Statistical Analysis

The study employed statistical tools to analyze the experimental data effectively. The mean was calculated by summing up all the data values within the collection and dividing the total by the number of data items present. For example, in an experimental setup involving six pots with varying compositions of Earth soil and Lunar soil (e.g., 40:60, 45:55, and 50:50 ratios), data such as soil pH, acidity, and plant growth measurements were collected bi-weekly. By calculating the mean, the study determined the average performance of each setup to provide a clearer understanding of the data trends. This approach ensures a representative central value, facilitating comparisons between different experimental conditions.

For the comparative analysis of results, simple correlation was utilized to identify the relationship between variables, such as the influence of soil composition, the type of light source (e.g., red/blue light or fluorescent light), and the application of human urine fertilizer on plant growth and principal component analysis for differentiation between the control and experimental groups, emphasizing the effectiveness of the experimental treatments in enhancing pea plant growth across multiple parameters. This enabled the study to assess how closely related changes in one variable (e.g., soil pH) were to changes in another (e.g., plant height). The use of such statistical methods provided a structured means of evaluating the experimental outcomes systematically and drawing meaningful conclusions about the effect of various treatments.

7. Results and Discussion

Over the course of our experiment, we collected data three times (bi-weekly) over the course of 6 weeks growing period. The experimental pots outperformed the control in average in terms of growth shown in table 1 and figure 2 below. The researchers believe since the human urine is rich in Urea, Nitrogen and Phosphorus thus help the growth of the plant. A very slight difference on control group but shows the effect of the fertilizer in all Pots (A, B and C).

Moreover, table 1 represents the growth responses of pea plant in different concentration influenced by human-urine fertilizer. As observed in variable group A with the concentration soil mixture of 40% earth soil and 60% lunar soil, the growth of the pea plant in week 1 measured with 3.8 cm, 9.5 cm, respectively, while the variable group B got the highest growth of pea plant with soil mixture of 50%-50% earth and lunar soil with 11cm, 12 cm, respectively in the first week. As figure 1 represents the comparative analysis of pea plant growth between control and experimental variable with different soil concentrations per week observation. As presented, B.1 with 50% earth soil, 50% lunar soil has 12 cm and 20 cm growth of pea plant that implies the highest growth compare to the other variables in week 1 and week 2, respectively. However, in week 3, B.2 got the highest mean growth with 20.67 cm compare to the other variables with stable increase of growth and the same time has the second highest growth with 30 cm in week 6 influenced by human urine fertilizer. This result implies that the fertilizer influences a positive response to the nutrient dynamics needed for the plants to grow and develop [7].

Growth Parameters ***(Growth in cm, Light Intensity in lux, pH and Moisture)***													
		Week 1				**Week 3**				**Week 6**			
Variables	*Concentration*	*Growth*	*Light I*	*pH*	*Moisture*	*Growth*	*Light I*	*pH*	*Moisture*	*Growth*	*Light I*	*pH*	*Moisture*
A.1 Control A.2 Experimental	40% Earth Soil 60% Lunar Soil	3.8	610	8	1.6	5	600	8	1.9	7	600	8	2
		9.5	600	8	1.9	14	600	8	1.9	28	630	8	2.1
B.1 Control B.2 Experimental	50% Earth Soil 50% Lunar Soil	11	600	7	2.0	18	600	7.5	2.5	31.1	600	8	3
		12	600	7	1.5	20	600	7	1.5	30	610	7	2
C.1 Control C.2 Experimental	45% Earth Soil 55% Moon Soil	8	620	7	4.6	11	600	7	5.0	21	600	8	5.5
		11	620	7	3.0	15.5	700	7.5	3.5	24	700	8	4

Table 1: Growth Responses of Pea Plant in Different Concentration Influence by Human-Urine Fertilize

In addition, A.1 with 40% earth soil and 60% lunar soil concentration as control variable has the least growth compare to the other variables with the mean growth of 5.2 cm and has 7 cm in week 6.

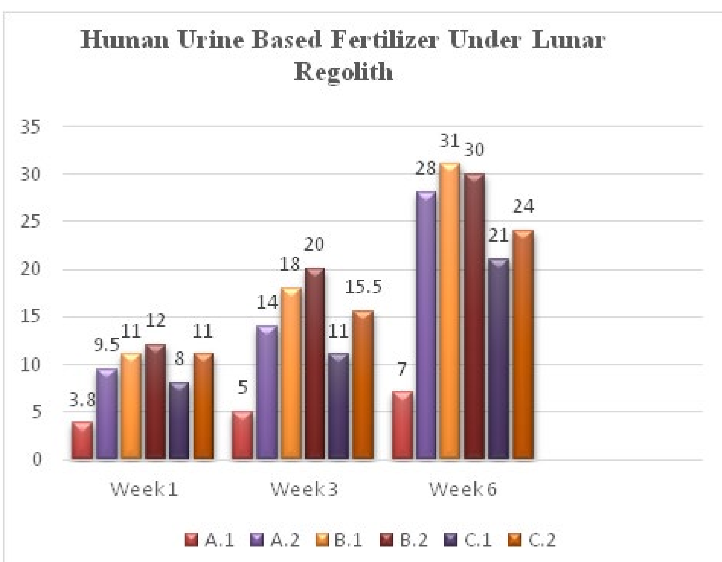

Figure 1: Comparative Analysis of Pea Plant Growth Between Control Group and Experimental Variables

Over and above that, B.1 with 50% earth soil, 50% lunar soil as control variable presents a high growth but slightly fluctuating result as presented in figure 1, it only means that pea plant has a slower rate development with regard on its soil composition [8]. Explained the relative relationship of nutrient dynamics that influences the growth of plants, it is mentioned that growth fluctuation is due to the slow nutrient cycling and fluctuations of photosynthesis which affirmed by the study [9].

On the other hand, the figure 2 presents morphometric responses of pea plants under different treatments, observed over six weeks. The plants were subjected to control conditions or experimental conditions, likely involving the use of human urine as fertilizer. Measurements were taken for leaf size, stem height, and root length at Week 1, Week 3, and Week 6. In Week 1, the control group with 40% earth soil and 60% lunar soil had leaves measuring 3 cm, stems 2 cm, and roots 5 cm, while the experimental group showed similar leaf size (3 cm) but significantly taller stems (10 cm) and longer roots (7 cm). The control group with 50% earth soil and 50% lunar soil had leaves at 4 cm, stems at 16 cm, and roots at 10 cm, whereas the experimental group displayed slightly larger leaves (4.5 cm), shorter stems (9 cm), but much longer roots (14 cm).

By Week 3, the control group with 40% earth soil and 60% lunar soil showed minor growth (leaves 3 cm, stems 5 cm, roots 7 cm). The experimental group saw moderate improvements (leaves 3.5 cm, stems 14 cm, roots 8 cm). The control group with 50% earth soil and 50% lunar soil had leaves at 4 cm, stems at 15.5 cm, and roots at 14 cm, while the experimental group demonstrated further growth (leaves 4.4 cm, stems 11 cm, roots 17 cm).

In Week 6, the control group with 40% earth soil and 60% lunar soil had leaves at 3.5 cm, stems at 10 cm, and roots at 8 cm. In contrast, the experimental group displayed no change in leaf size (3.5 cm) but significant stem growth (26 cm) and moderate root growth (9 cm). The control group with 50% earth soil and 50% lunar soil reached leaves at 4.5 cm, stems at 30 cm, and roots at 17 cm, while the experimental group showed the most notable growth with leaves at 5 cm, stems at 24 cm, and roots at 20 cm.

Overall, the experimental groups, likely treated with human urine, exhibited enhanced growth compared to the control groups. This was particularly evident in stem height and root length, suggesting that human urine can be a potent fertilizer, promoting substantial vegetative growth and root development in pea plants.

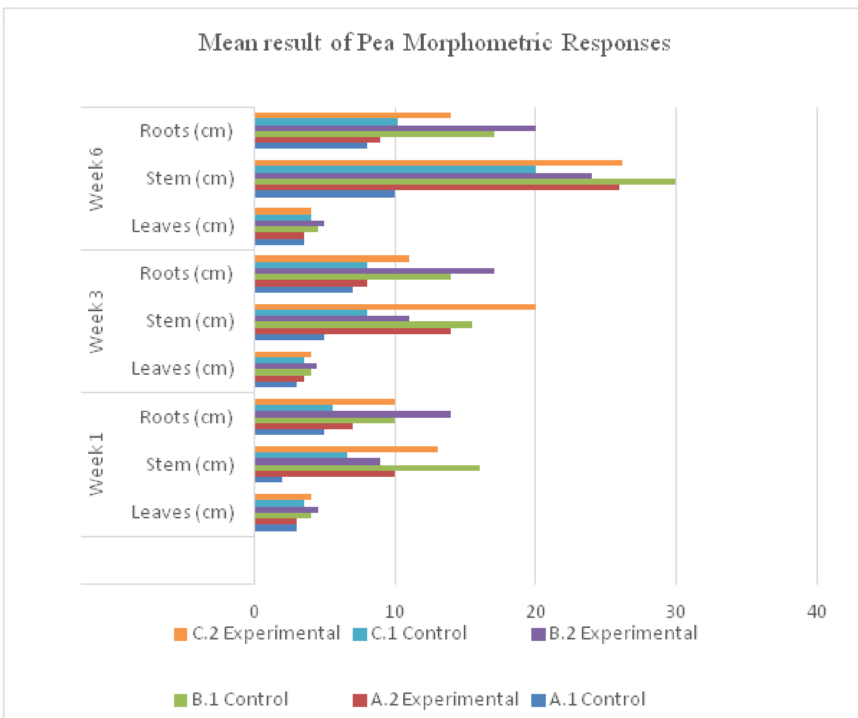

Figure 2: Morphometric Responses of Pea Plants Under Different Treatments, Observed Over Six Weeks

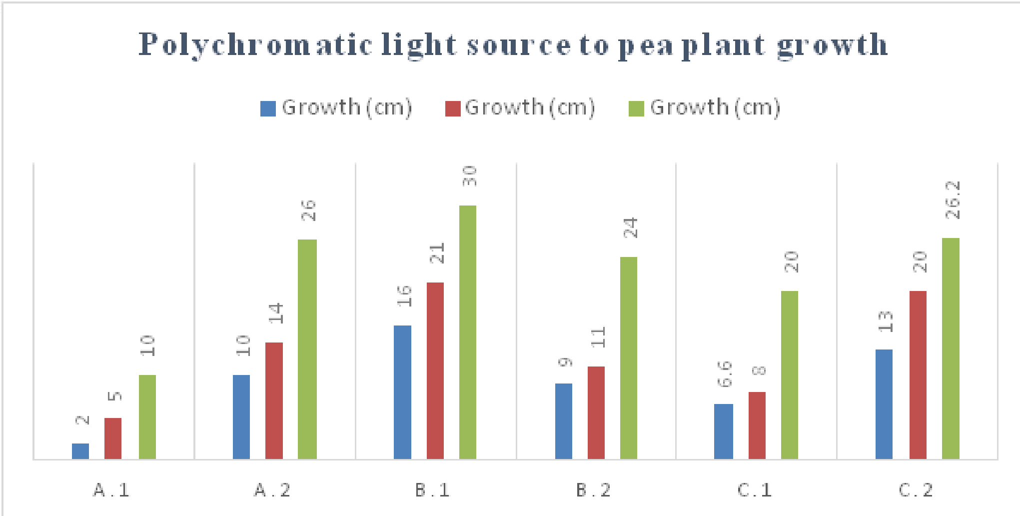

Figure 3: Growth of Pea Plant in 6 Weeks' Growth Period Under Lunar Regolith in Different Concentration in Control and Experimental Treatment (Polychromatic Light Source)

The figure 3 presents data on the growth of pea plant in 6 weeks' growth period under lunar regolith in different concentration in control and experimental treatment (polychromatic light) exposed to red, blue and white light. Each experimental condition is denoted by a specific variable, detailing the concentration of earth and lunar soil, along with measurements of growth, light intensity (Lux), pH levels, and moisture content. While the excellent growth was observed, a complete 8 weeks should be observing. The lack of 2 weeks is due to the replanting.

Notably in figure 3, the growth trends vary across the different conditions. For instance, in the control condition A.1, where the soil composition consists of 40% earth soil and 60% lunar soil present in table 2, plants exhibit modest growth, starting at 2 cm in Week 1 and reaching 10 cm by Week 6. Conversely, the experimental condition A.2 shows significantly higher growth rates, with plants reaching 10 cm in Week 1 and expanding to 26 cm by Week 6. Similarly, control condition B.1, featuring a 50-50 mixture of earth and lunar soil, demonstrates substantial growth, while the experimental counterpart B.2 exhibits slightly lower growth rates but still shows notable development.

Moreover, C.2 with 45% earth soil and 55% moon soil exhibits potential influence by polychromatic light source compare to C.1 with slightly slower growth rate but still performs development. The data underscores that the influence of polychromatic light source to plant growth and composition considering with variations in light intensity, pH levels, and moisture content also observed across different conditions. Overall, B.1 has the highest rate of plant growth compare to the other without the influence of light, however, with 45% earth soil and 55% moon soil exposed to light shows also an exponential growth compare to other groups as presented in table 2. These findings contribute to a deeper understanding of the factors impacting plant growth and environmental conditions in experimental settings.

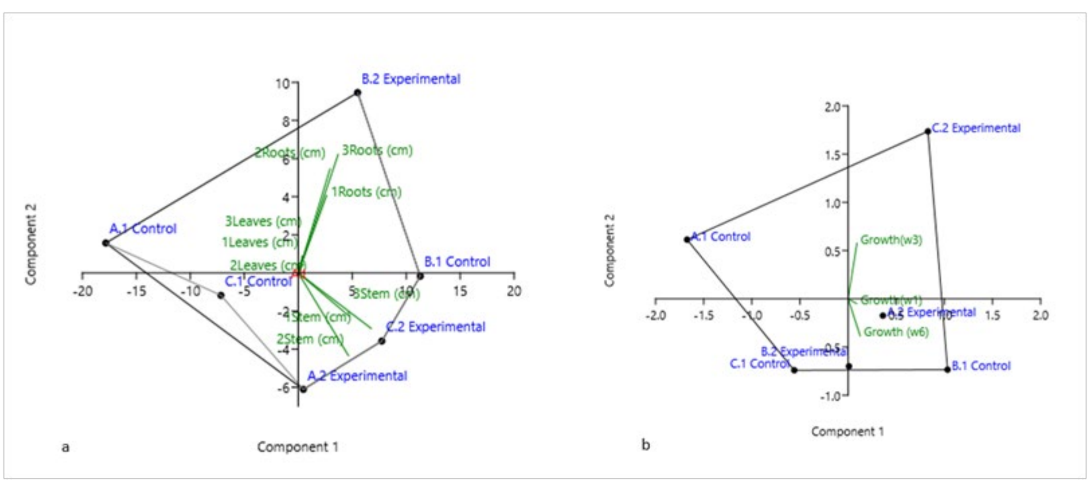

Figure 4: Principal Component Analysis (a) Utilization of Human Urine Fertilizer, (b) Polychromatic Light Source

Figure 4 presents the plot illustrates a principal component analysis (PCA) biplot both the utilization of human urine fertilizer and polychromatic light source, highlighting the relationships between experimental and control groups based on the growth parameters of pea plants. In figure 4a, the data points represent different plant parts (roots, stems, and leaves) measured in centimeters, along with their contributions to the two principal components (Component 1 and Component 2). The control groups and experimental groups are distributed across the axes, reflecting their distinct growth patterns.

The green vectors indicate the growth parameters (roots, stems, leaves) and their correlations with the principal components. Longer vectors suggest a stronger contribution of the corresponding parameter to the variation in the dataset. For instance, stem appears to contribute significantly to Component 1, whereas roots and leaves contribute to both components with varying magnitudes.
In figure 4b, the green vectors indicate the contribution of plant growth across weeks to the variability captured by the principal components. The direction and length of the vectors reveals their relative influence. Growth measurements for week 6 suggesting it has a significant impact on distinguishing between experimental and control groups.

The experimental groups are positioned further along the positive side indicating improved growth outcomes compared to the control groups. This separation suggests that the experimental conditions, which likely involve urine-based fertilizer and polychromatic light, had a positive impact on plant development. In contrast, the control groups cluster closer to the negative side reflecting lower growth rates under standard conditions. Overall, it demonstrates clear differentiation between the control and experimental groups, emphasizing the effectiveness of the experimental treatments in enhancing pea plant growth across multiple parameters.

8. Conclusion and Recommendation

In conclusion, the comparative analysis of pea plant growth under different soil concentrations reveals distinct patterns over time [10-15].

With 50% earth soil, 50% lunar soil initially exhibits the highest growth in weeks 1 and 2, while with 50% earth soil, 50% lunar soil influenced by urine-based fertilizer surpasses other variables by week 6, indicating stable growth throughout the observation period. This stability suggests that the fertilizer used in 50% earth and lunar soil positively influences nutrient dynamics crucial for plant growth. Conversely, A.1, with a higher concentration of lunar soil, shows the least growth overall, while B.1, with equal parts of earth and lunar soil, displays high but fluctuating growth, suggesting a slower rate of development.

In addition, the effectiveness of human urine-based fertilizer and polychromatic light in enhancing pea plant growth significantly influence the variability, with stems strongly contributing and roots and leaves contributing to both components. The impact of growth across weeks, with week 6 showing the most substantial influence on separating experimental groups from controls. Experimental groups consistently outperformed controls, as indicated by their position along the positive components, demonstrating superior growth outcomes under experimental treatments.

The measurement of plant height throughout the growing period supported the original hypothesis, indicating that the experimental group exposed to polychromatic light source generally exhibited better growth compared to other groups. It was observed that the group exposed to polychromatic light source with a composition of 40% earth and 60% lunar soil exhibited exponential growth, with plants reaching 10 cm in height in Week 1. This highlights the potential benefits of polychromatic light source in promoting plant growth, particularly in combination with specific soil compositions. These findings highlight the impact of nutrient dynamics on plant growth and attribute growth fluctuations to slow nutrient cycling and variations in photosynthesis. Overall, the study underscores the positive influence of using human urine as fertilizer and potential benefits of polychromatic light source of soil composition and nutrient dynamics in influencing pea plant growth and development.

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