THESIS

EVALUATION OF THE EFFECTIVENESS OF SUPPLEMENTAL LIGHTS VS NO SUPPLEMENTAL LIGHTS ON HYDROPONICALLY GROWN LETTUCE

Submitted by

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ABSTRACT

EVALUATION OF THE EFFECTIVENESS OF SUPPLEMENTAL LIGHTS VS NO SUPPLEMENTAL LIGHT S ON HYDROPONICALLY GROWN LETTUCE

The purpose of the study was to examine literature from the past 20 years regarding the evaluation of the Effectiveness of Supplemental lights vs No supplemental lights on Organic and Synthetic lettuce production via hydroponically growing lettuce in a greenhouse. The two types of lettuce are 1) green salad bowl and 2) gourmet blend mix. This research was conducted in the Colorado State University, Ft. Collins (CSUFC). The Researcher used quantitative research design with basic agricultural, horticultural, quantitative, and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) quantitative statistical calculations. This research method addressed agricultural horticulture research findings from agriculturalists, farmers, horticulturalists, policy makers, researchers, scientists, universities, and/or other key stakeholders in the agriculture, farming, greenhouse, and horticulture industry. The student researched the historical and current literature and the effects of altering the Supplemental lights for the maximum growth and development of healthy mineral rich lettuce. Twenty-three minerals were tracked and measured using the ICP-MS after production via Supplemental light vs. No Supplemental light using parts per million (ppm) converted from mg, (ng/g), and other amounts. This Thesis contains five chapters including: (1) Introduction, (2) Literature review, (3) Material and Methods, (4) Results and Discussion and (5) Conclusion. Finally, research recommendations are made for future replications and studies to accentuate and increase the validity and reliability of this study.

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Agriculture is a noun and is defined as the science, art, or occupation concerned with cultivating land, raising crops, and feeding, breeding, and raising livestock; farming. The production of crops, livestock, or poultry (Dictionary.com, Agriculture 2016).[iii]

Gourmet Lettuce is defined, according to burpee.com (2016) as a popular loose-leaf blend that is selected for a long harvest. The full description features a tasty, colorful, and assorted mix of five loose-leaf lettuces with varied green to red shades and textures from smooth to ruffle. It is a lettuce that has a tolerance for hot weather and a long harvest season from early spring to a second or third fall crop.

Green Salad Bowl Lettuce is defined by seedaholic.com (2016) as Salad Bowl Lettuce includes both red and green varieties. Aptly named, one plant fills a salad bowl with no need to add other lettuces. 'Green Salad Bowl' is a large fast-growing Rosette Lettuce with long, light green and deep cut leaves. It was introduced in 1952 and was an All-America Selection winner in the same year. It produces large, slow bolting, non-heading plants with sweet, succulent green leaves. It is successional every 7 to 10 days from March to August for harvesting April to October. If regularly harvested, individual leaves and Green Salad Bowl will keep producing right to the end of the season.

Horticulture is defined at as the cultivation of a garden, orchard, or nursery; the cultivation of flowers, fruits, vegetables, or ornamental plants and the science and art of cultivating such plants (dictionary.com, Horticulture, 2016)

Hydroponics is defined by dictionary.com (hydroponics, 2016) as cultivating plants with the roots in liquid and nutrient rich solutions instead of soil or soilless growth of perennial and other plants.

Hydroponics is defined by Merriam-Webster (2016) as growing plants in nutrient solutions without or with soil or other mediums for mechanical or stem support.

Light-emitting diodes (LEDs) according to Massa and Wheeler

(2010), plant productivity in response to Supplemental lighting defines LED's as small Light-emitting diodes (LEDs) with durable, long lifetimes, and which emits minimal heat, and give the grower or user the option / ability to alter and select multiple wavelengths.

Another definition of LEDs (2016) states that a light-emitting diode (LED) is a semiconductor

that emits light when electricity passes through it. It emits a soft monochromatic, single wavelengths with output ranging from red at 700 nanometers to blue-violet at 400 nanometers. There are also LEDs that emit infrared (IR) energy at 830 nanometers or more and it is considered (named) an *infrared-emitting diode* (IRED) but is not the topic of this research.

Independent Variable, according to the National Center for Educational Statistics, (IV, NCES, 2016, Footnote) “a standalone variable that's not changed by other variables being measured.” **Dependent Variable** is something that depends on other factors. For example, a test score could be a dependent variable because it could change depending on several factors such as how much you studied, how much sleep you got the night before you took the test, or even how hungry you were when you took it. Usually when you are looking for a relationship between two things you are trying to find out what makes the dependent variable change the way it does. Independent variable causes a change in Dependent

Variable and it isn't possible that Dependent Variable could cause a change in Independent Variable.

RESEARCH PROPOSAL

Successfully growing Hydroponic Organic Lettuce (*Lactuca sativa)* in greenhouse

settings under Supplemental lights vs no supplemental lights or normal sunlight can fill marketing needs in Colorado, the Middle East, and dry and arid regions globally. The purpose of this proposal was to research and test how to successfully grow two types of Lettuce 1) green salad bowl and 2) gourmet lettuce under Supplemental lights vs. No Supplemental lights with a high traceable mineral content in six weeks using hydroponic methods. It tested the effectiveness of Supplemental lights vs. No Supplemental lights on quality, quantity, of lettuce production in the Colorado State University, Ft. Collins (CSUFC).

After six weeks of growth, the Researcher (Fatima Al-Houti) proposed a mineral content type where the concentration of nitrogen shall be measured using the Induced Coupled Plasma Mass Spectrometry (ICP-MS). This tested the content of 25 high minerals in eatable lettuce and tested the mineral content with ICP-MS Machine and the ICPMS Methodology. Thus, the Independent Variable (IV) was the Supplemental lights vs No supplemental lights and the Dependent Variable (DV) was the lettuce with rich mineral contents. The researcher proposed and showed that the two types of lettuce could be successfully grown hydroponically in a CSU. Greenhouse and tested for minerals. The findings and this methodology can later be generalized to other arid and desert areas globally where there is a critical need. Fertilizer was changed to a DV in this study. The tracking, use, and measurement of water and the vitamin content are not a part of this research.

This proposal examined the key literature and research from the past 20 years and major historical articles from major food, agricultural, and horticultural theorist in the field who theorized, plant, cultivate, grow, harvest food for human consumption in controlled environments and govern nutritional values. First, this thesis proposal addressed the historical factors of greenhouse lettuce and food production. Second, the proposal examined the research findings of studies and several theories that relate to the cultivation of mineral rich lettuce in a controlled greenhouse horticulture environment. Finally, this proposal showed the critical need for the study itself and draws clear conclusions regarding the body of research, finding, and conclusions. This proposal included a five-section research study and literature review as follows: (1) Introduction, (2) Literature Review, (3) Materials and Methods, (4) Results and Discussion and (5) Conclusion.

CHAPTER 1: INTRODUCTION

All plants need various forms and levels of light to grow to full maturity and provide the minerals needed for maximum maturity and marketability. Without light, plants become undeveloped or poorly developed perennials that may or may not be suitable for sale and human consumption in various settings. However, modified agricultural and horticultural practices, conditions, lights, and other variables can assure maximum growth and usability. The lettuce variety *Lactuca sativa* is a much-desired food source with a critical need for better growing methods in arid climates where water is at a premium. There is a lucrative market with high profit potential in markets, restaurant, and homes use globally. The United States Department of Agriculture (USDA) British, Kuwaiti, other government globally affirm that there is a critical need for better growing methods in regions where water is at a premium and the sunlight is too hot for crops to grow without massive water evaporation. The USDA also confirms that there is a critical need for millions of heads of lettuce with the increasing population growth worldwide. Plants that grow in light or open sunlight vs. a controlled Supplemental light and agricultural and horticultural conditions can be harder to grow, restricted in the times to grow the plants, length of the growing seasons, and other factors that reduce the amount produced and marketed in arid and tough growing areas. Increased lighting and improved agricultural and horticultural growing conditions can greatly improve the amount of lettuce produced in the world and the arid areas where water and other ideal growing conditions do not exist.

Background and overview of the problem

Globally, all plants need various amounts of light for maximum growth and lettuce is one of the more delicate plants that must be exposed to the proper amount of light and not too much. lettuce plants also need the right amount of water, restricted use of chemicals, and proper care for human consumption. According to the United States Department of Agriculture (USDA) Economics, Statistics and Market Information System, 72, 178 million pounds of lettuce were produced, sold, and utilized in other ways in 2010. In (Table 1) Head and other lettuce: U.S. monthly export volume, 1990-2010, the government showed the critical need for lettuce globally. Successfully growing Hydroponic Organic Lettuce *Lactuca sativa* in greenhouse settings under Supplemental lights vs. No Supplemental lights or normal sunlight can fill marketing needs in Colorado, the Middle East, and dry and arid regions globally. However, there is a critical need for efficient, new, controlled, and profitable methods to meet the public demands for lettuce. Hydroponically grown lettuce in greenhouses under Supplemental lights vs No supplemental lights is one methodology for improving the crop yields and success of lettuce production. This method can fulfill the need to control the environments and produce lettuce throughout the year in a manner that has never been equaled globally. Thus, utilizing different forms of lights is worth investigating to determine if Supplemental lights vs. No supplemental lights can aid in improved lettuce growth with less trouble. Light becomes more important in the growing of lettuce and we need to determine if Supplemental lights vs. No supplemental lights have a significant effect on the quality and mineral contents of the final plants. Plants exposed to Supplemental lights may grow in less risk than in sunlight and improve the global production of lettuce worldwide under improved agricultural and horticultural conditions.

Statement of the problem and research

There is a critical need for higher, vegetable, production in the world in all desert and arid countries where water is scarce but the focus of this research is lettuce growth. The world population is increasing daily and many underdeveloped cities and countries in the Middle East, Africa, India, and other desert or arid areas need to conserve and better use water and artificial supplemental lights to increase their lettuce and food production as all the authors agree. First, this thesis proposal addressed the historical factors of greenhouse lettuce and food production. Second, the proposal examined the research findings of studies and several theories that relate to the cultivation of mineral rich lettuce in a controlled greenhouse horticulture environment. Finally, this proposal showed the critical need for the study itself and draws clear conclusions regarding the body of research, finding, and conclusions. The researcher used the Lettuce *Lactuca sativa or G*reen Salad Bowl and Gourmet Blend Mix Lettuces in the Colorado State University horticultural greenhouse environment. This required alternative environments that diverts growers away from the conventional soil approach using synthetic and organic plants.

The key literature and preliminary research findings from the past 10 years for major quality of food in the field who theorized regarding the dependent and independent variables, plant, cultivation, grow, and harvesting of food for human consumption in controlled inside environments were examined. It included the examination of field nutritional status methods by various researchers to determine the mineral and vitamin compositions of the water and soil, Chlorophyll and Nitrate Nitrogen Analysis used by Karla, (1998), Lairon. D. (1986), Raven. P. (1992), and Succop, C. (1998), and final Mineral Vitamin contents.

Significance of the study

This research method addressed agricultural horticulture research problems and findings from agriculturalists, farmers, horticulturalists, policy makers, researchers, scientists, universities, and/or other key stakeholders in the agriculture, farming, greenhouse, and horticulture industry. The student researched the historical and current literature and the effects of altering the Supplemental lights for the maximum growth and development of healthy minerals rich in lettuce. Twenty-three minerals were tracked and measured using the ICP-MS after production via Supplemental light vs. No Supplemental light using parts per million (ppm) converted from mg, ng/g, and other amounts. The rationale of the study is to compare the effect of various amounts of Supplemental lights vs No supplemental lights on lettuce production and mineral retention. The findings show that growing lettuce in a controlled greenhouse environment using hydroponic technology and the best agricultural and horticultural environment with supplemental light allow more control of the growing and production environment. It provided a basis for future research and improved practices for hydroponically growing lettuce in a greenhouse environment using Supplemental lights vs No supplemental lights can increase the amount of lettuce produced, growing periods, control by the growers, and other negative factors generic to growing in open sunlight or natural light. The Supplemental light options have numerous benefits to the harsh conditions of open fields and growing areas that are exposed to changing conditions.

Researcher perspective

Finally, this research showed the critical need for the study itself and draws clear conclusions regarding the body of research, finding, and conclusions. This proposal included a five-section research study and literature review as follows: (1) Introduction, (2) Literature Review, (3) Materials and Methods, (4) Results and Discussion and (5) Conclusions. The researcher believes that there is a critical need for greater lettuce production in general and using Supplemental lights vs. No supplemental lights or outside sunlight in arid areas and controlled greenhouse climates where water and light can be controlled. However, the focus of this study is not the water but Supplemental lights vs. No supplemental lights variables. The Null Hypothesis of this study is that there is a significant effect upon mineral content of lettuce grown under Supplemental lights vs. No supplemental lights with fertilizer in a greenhouse agricultural and horticultural environment. The primary horticultural research but ultimately modifiable inside Supplemental light environments were used and modified. This objective can be achieved by using the following sub objectives:

1. Change the historical method of greenhouse lettuce production.

2. Examine the research findings of studies and several theories that relate to the cultivation of mineral rich lettuce in a controlled greenhouse horticulture environment or the CSU P.E.R.C. Greenhouses.

3. Compare hydroponic greenhouse lettuce production using Supplemental lights vs. No supplemental lights using varied conditions.

4. Determine if 25 different minerals in 76 heads of lettuce can be successfully produced for human consumption.

5. Determine if hydroponically grown lettuce can be successfully produced and marketed in mass to meet the growing demand Vs traditional methods for growing lettuce in outside light and water sources.

6. Use ICP-MS Sample Digestion Materials to measure digestibility.

7. Draw other conclusions.

CHAPTER 2: LITERATURE REVIEW

“the scientific name of lettuce is *Lactuca sativa. Lactuca* means 'milk-forming', *sativa* means 'common'. It is related to over one hundred wild species of *Lactuca according to encyclopedia.* The varieties of Data on lettuce varieties produced in greenhouse settings is needed by local reginal, and other global growers. Small, and larger farmers to meet the growing demand in arid, extreme, and very dry climates for this delicate crop. To date, more Arabic and Colorado growers are focusing on growing lettuce inside to control the growth factors, water, and effects of sunlight, arid climate, and effects of harsh and varied weather conditions. Of the over one hundred wild species of *Lactuca* sativa, the two types of lettuce used in this research were the (1) Green Salad Bowl Lettuce and (2) Gourmet Blend Mix Lettuce. The sources used for this research were peer and public open review, government, USDA, American, Australian, British government, online and /or other agricultural, horticultural, farm, policy makers, researchers, scientists, universities, and other valid research.

Purpose of literature review

The purpose of this literature review is to show the critical need for this research and the topic of Supplemental lights vs. No supplemental lights and its affect upon the growth of marketable lettuce and its retention of the 25 minerals tracked in the processes. According to New Mexico State University (2016), approximately four million tons of lettuce was produced by the states of California, Arizona and New Mexico in 2011 and they are the two top producers among the top five lettuce producing states. The lettuce production in the United States in 2010 was 72,178,000 tons according to the USDA (2011). More recently, the numbers and demand for lettuce has increased even more.

Idso, C. of the *Center for the Study of Carbon Dioxide and Global Change,* 15 June 2011, in the co2science.org (2011) showed the need for lettuce by 2050. The Agricultural Marketing Resource Center (agmrc.org, 2011) shows that there will be an increase by 1/4 of all lettuce produced and marketed for human consumption. The United States Department of Agriculture (USDA) Economics, Statistics and Market Information System claims that 72, 178 million pounds of lettuce were produced, sold, and utilized in other ways as of 2010. This Production is generally in considered a cool-season crop between 73ºF and 45ºF, however, hydroponic greenhouse practices are changing this production cycle to a more productive time period (s) where it will be available throughout the year globally. Farmers, families, and companies will be able to select more specific time limits and harvesting periods. He confirmed that California and Arizona are at the top of the production list in the United States with California producing 71 percent of the head lettuce in the US in 2013 and Arizona about 29 percent and the two states grow about 98% of the leaf lettuce nationwide.

According to the USDA, in their 2012 updated Census of Agriculture, lettuce occupied 323,359 acres and increase of 3% since 2007 (USDA, 2012). The number of farms increased by 38% from 2007 and 2012 and the demand is still high. In California, the tonnage for lettuce was as follows per acre: 20 tons' iceberg, 15 tons for romaine, and 12 tons for leaf lettuce. According to their calculations, the overall US production in 2014, the latest figures in this research, was 3,881 million pounds, about 12% of that amount exported to other countries, islands, and 169.7 million pounds imported to the US to keep up with demands. This accounted for a profit of $1.5 billion making it the most lucrative crop in the US. According to the Commodity Profile: *Lettuce* by Hayley Boriss (aic.ucdavis.edu, 2005; fao.org, 2005; and fao.org/docrep, 2005), these numbers are accurate and they affirm these numbers and the demands.

Dickerson (2016) of New Mexico State University (NMSU) showed that Greenhouse Vegetable Production via hydroponics is in higher demand and is increasing in the Midwest especially during the non-summer months. He showed that the Greenhouse's location, construction, temperature control, soil culture, hydroponic culture, crops and culture, carbon dioxide enhancement, integrated pest management, and other factors are all very important. According to Dickerson, lettuce prefers low daylight temperatures at about 60º-65ºF and a night temperature of about 50º-55ºF for the fall and spring crops. If the temperatures are too high in the greenhouse or too low, they can damage lettuce. Generally, it takes 30 days for the leafy lettuce to grow from seed to appropriate size for transplanting them to permanent locations. Dickerson affirms that it takes 12-15 weeks during the winter months and 8-10 weeks during the spring months to grow. They confirm that lettuce feeds poorly and needs normal soil, fertilizer, and/or hydroponic care for proper growth. Leaf and Bibb lettuce are most acceptable but all types of lettuce will grow successfully in a hydroponically controlled environment.

Critical need for more food globally

Yahia, E. (2005) states that Post Harvest Technology in the Near East and North Africa (NENA) regions regional growing differences exist but millions of acres of lettuce are grown in countries with drastically varied GNP's: $19,020 in Kuwait, Sudan $330, $350 in Yemen, $470 in Pakistan, $1,500 in Jordan, $3,700 in Lebanon, and $17,870 in the UAE. He confirms that regional cooperation in growing food could greatly impact the food gaps. However, research in the areas, especially in the NENA region is scarce confirming that there is a need for quantitative research and crop issues. In these NENA and other regions, it is commonly known that more than 40,000 people starve to death a week due to a lack of food and thus this research is extremely valuable in underdeveloped and war-torn regions. There is a critical need for more food in the areas where about 35 Million metric tons (mmt) of cereals was grown in 2010 but

55mmt were consumed. There is a need for 80mmt of fruit and vegetables and only 53mmt were produced in 2010. Agricultural trade balance in the Maghreb sub-region is negative. Thus, there are negative balances in food in numerous countries globally and some import from 30 – 60% of their food. Various researchers studied field nutritional status methods to determine the mineral content of the soil and plants. Various online sources show that it takes 1000's to millions of acres to grow lettuce in uncontrolled environments such as mlive.com (2010) quoting Hogan, J. (2017), gardenersnet.com (2010), Blake, C. (2007), Thomasnet.com (2010), Huang, M., Li, M., Rutter, J., Walters, J., Wiwattarangkul, P. (2016) and Asknumbers.com (2009) show there are millions of acres used to grow lettuce and other crops for human and animal consumption and the numbers are increasing. Lettuce can be priced at about 1- 249 cases $2.18 per head to 1250 can be as low as $1.82 / head of lettuce.

Lights and its affect upon lettuce growth and retention of minerals

According to the Urbonaviciute, *A., Pinho, P., Samuoliene, Duchovskis, P, Vitta, P., Stonkus, A., Tamulaitis, G., Zukauskas, A., and Halonen, L.* (2007) and the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture (2007) the length of light rays effects on growth and development of lettuce, its maturation processes, growth, and nutritional qualities. It also affects the nitrites and sugars in the plant. The results of this study showed that lettuce can be affected by Supplemental lights and No supplemental lights variables and when those lights are modified and the length of the lighting exposure is extended or decreased, it alters most qualities of the plant. Urbonaviciute, A., et al (2007) also proved that it affected carbohydrates. In support of this research, pfaf.org (2016) states *Lactuca sativa* has nutritional, medicinal, and other valuable qualities. It is an annual/biennial that is short by nature at 0.9 m (3ft) by 0.3 m (1ft in), and was probably one of the first perennials transferred to America from the UK where it grows hardy and is not frost sensitive or tender. Generally, it flowers in the late summer months of July to August but the seeds reach maturity from Aug to September when grown in outdoor climates. It is a hermaphrodite plant with male and female organs and is pollinated by flies or self-fertilized. It can grow in slightly light (sandy) to medium (loamy) soils but it prefers the soils to be well drained and not waterlogged. It also grows well in neutral and basic (alkaline) soils but can grow with or without shade in moist soil. Globally, the milky substance in lettuce is considered valuable and hence increases the need, marketability, and global demand for the product.

According to Khairy, H. and El-Sheikh, M. (2014) the mineral contents of plants are affected by the amount of light they receive or do not receive. In section 3.5. (Minerals composition) of the article, the authors affirm that Sodium (Na), Potassium (K), Calcium (Ca), and Magnesium (Mg) are among the minerals affected by the light sources. Thus, the mineral contents varied according to the light, light wave exposure, seasons of the year, environmental factors, physiological factors of the plant, and mineralization practices. They also confirm that light and other factors affect calcium levels in the plant, Na, K, Ca, Mg, and the contents of these macro elements, though in small amounts, the mineral contents varied by the seasons. The authors in another study, Hecher, E., Falk, C., Enfield, J., Guldan, S. and Uchanski, M. (2014) explained the importance of greenhouses used in this study are called Low-Cost High Tunnels or Hoop Houses with controlled environments, lighting, hydrophonics, and other variables. In the Economics of Low-cost High Tunnels for Winter Vegetable Production in the Southwestern United States they showed the need for Low-cost greenhouses in the Southwestern United States where farmers, small farmers, and families can better control the lettuce, risk, extend and alter the growth season, simulation models, and crops. They firmly established the need for these low cost hydroponic houses with controlled Supplemental lights vs. No supplemental lights to effectively grow lettuce. They affirm there is a critical need and little valid and in-depth research from the past 10 years regarding Supplemental lights vs. No supplemental lights in the Midwestern United States for small scale farmers. This study specifically focused on *Lactuca sativa*. This was a Single Layer (SL) and Double Layer (DL) study of lettuce and spinach. This study also confirms that the cost factors makes Hydroponic greenhouse growing profitable. In the sensitivity analysis for lettuce growth, the researchers confirmed that the SL design was more profitable than the DL design for lettuce where a group of lettuce plants has another group or layer under it.

This process of growth was thought to be more efficient but it was not. The lettuce growing and the projects were more successful than lettuce grown in the open, arid, and dry sunlight and direct heat. Likewise, it gave farmers more flexibility in planting in that they determined that the planting date were not a factor in yield, profit, and success in the greenhouses, the farmers / growers were free to select their own planting dates, times and seasons with much flexibility. The result was higher yield with lower cost for the SL lettuce production grown in hydroponic greenhouse with Supplemental lights vs. No supplemental lights.

Seasonal extensions of growing seasons

This research showed there can be random and personal changes to the growing seasons in greenhouse scenarios at a person's discretion and choice. This makes it more inviting, manageable, and profitable for farmers or families to manage their profits and losses when raising lettuce in a greenhouse setting with Supplemental lights vs. No supplemental lights and lower the risk of crop losses (Hecher, E., et al, 2014). The Hoop House Project (Guldan, S., 2012) confirms this. It affirmed that other researchers agreed with the findings that Hydroponic Greenhouse Farming of lettuce with Supplemental light s vs. No supplemental light s or Regular

Light or Direct Sunlight in arid climates where there is little water and little control of external factors was far more successful:

"Chelsea Green Publ., White River Junction, VT. Conner, D.S., K. B. Waldman, A.D. Montri, M. W. Hamm, and J. A. Biernbaum. 2010. Hoophouse contributions to economic viability: Nine Michigan case studies. HortTechnology 20:877–884. Enfield, J.S. 2012. Winter production of leafy greens in the southwestern USA using high tunnels. MS Thesis, New Mexico State Univ., Las Cruces ".

Supplemental lighting vs. no supplemental lighting research

According to purl.fdlp.gov (2010), preparation and transitioning to Supplemental light vs. No supplemental light with Hydroponic Greenhouse Models required a hearing before congress of the United States. Thus, the transition is and has been expensive due to the political red tape but it is a transition that needs to take place for desert and arid areas of the United States and small farmers / families to grow lettuce with minimal cost. In this research and others, the NCES (2016) used Supplemental lights vs. No supplemental lights as the Dependent Variables for growing lettuce. Likewise, Resh, H. (2012) and the National Center for Educational Statistics (NCES, 2016) investigated Independent Variables (IV) and Dependent Variables (DV) to determine the validity and reliable facts regarding the Supplemental lights vs. No supplemental lights. The success or findings of Hydrophobic Greenhouse experiments and mineral retention in lettuce was clear. According to the NCES, the IV, Supplemental lights, stands alone, is not altered, changed by the DV (Lettuce) or other variables one is measuring. This shows that the Hydroponic food production for the home gardner and larger commercial hydroponic growers has tremendous ramifications globally in the restructuring of when, where, and how food is grown and marketed at minimal cost and varied amounts. This process of using Supplemental lights vs. No supplemental lights was used by White, T. (2014) as a successful prototype for growing food under Supplemental lights in an environments and high-rises, skyscrapers, and giant barges. They confirmed that the amount of Supplemental lighting vs. No Supplemental lighting can cause a change in the DV (lettuce quality, size, etc.) and it isn't possible that Dependent Variable (lettuce) could cause a change in the IV (Supplemental lights) (NCES, 2016, p. 1, [8]) and those changes can last up to 10 years according to previous researchers.

Plant productivity in response to early testing of supplemental lighting

According to Massa, and Wheeler (2010), plants responded to light-emitting diodes (LEDs) and have tremendous potential for lettuce and other crop production. The benefits of the Supplemental light vs. regular light, traditional lighting, sunlight, no Supplemental light, and other forms outweigh the negatives. The output of the narrow LEDs (single color, no phosphorcoated) vs. the traditional sources of electricity and the ability to alter it is essential and profitable for certain crops. The Supplemental lights can be blue, red, white, and/or a combination of all three and each has its benefits and restrictions according to this research and that of all the other Supplemental researchers. This article reported data from more than 30 researchers showing Supplemental lighting is valid and reliable sources vs. No supplemental lighting or Regular light, Sunlight, and other traditional sources. They confirmed too that it is less expensive, controllable, and the planting, growing, and harvesting timelines can be altered. See the references below: "(Bula et al. (1991) at the University of Wisconsin ….. blue light (Hoenecke et al., 1992)....aboard NASA's Space Shuttle (Barta et al., 1992) ….seedlings (Morrow et al., 1995), potato (*Solanum tuberosum* L.) leaf cuttings (Croxdale et al., 1997), *Arabidopsis thaliana* (Stankovic et al., 2002), and soybeans [*Glycine max* (L.) Merr] (Zhou, 2005)." More than 50 researchers addressed the various benefits and dynamics of using other types of Supplemental Light Red, Blue, Green, and White vs. No Supplemental lights. The purpose of this research and literature review is not to address the various types and colors of Supplemental lights, waters, soils, and non-Supplemental light variables. Fertilizer was changed to a DV in this study. The purpose is to examine supplemental light vs. no supplemental light or regular light in the growth of hydroponic greenhouse lettuce. The findings reported by Massa and wheeler (2010) affirm that Supplemental lights can be harnessed, used, modified, altered, and in other ways controlled to show their superiority over No supplemental light, Regular light, and uncontrollable sunlight. Okamoto, *K., Yanagi, T., Takita, S., Tanaka, M., Higuchi, T., Ushida, Y. and Watanabe, H.* (1996), while beyond the 10-year time limit for this research, introduced one of the key apparatuses that proves that Supplemental lights contribute to the photosynthesis of plants. Several of the best research findings, discoveries, theories, and articles regarding horticulture, agriculture, and lighting are outside the 10-year limit for current research. They introduced the "LED PACK, BIOLED, UNIPACK, and COMPACK" machines that were vital in proving the impact that Supplemental lights have on the overall functioning of the plants and their growth, retention of minerals, and other nutrients from the soil or water. One of their methods for proving this was the use of the exact type of lettuce in this study.

The purpose of their study was to introduce the plant growing apparatus that used Supplemental light and to evaluate No Supplemental light sources for normal and not defective plant growth. Lettuce seedlings were used and (*Lactuca sativa)* hydroponically grown for 14 days and growth successfully accelerated. Thus, Supplemental lighting vs. No Supplemental lighting in producing lettuce and other crops can be the solution to the global population crises where starvation is by product of that explosion and have a positive effect on reversing starvation statistics globally. As a result of these findings, the following normal areas for planting are reduced drastically in favor of reducing this space and controlling the elements with hydroponic greenhouses with Supplemental lights vs. No Supplemental lights as the only or primary light source:

1. Asknumbers.com (2009) show there are 43, 560 square feet in an acre and one square ft holds four head of lettuce. However, if you separate the rows or plantings by three feet and have only 2 heads per 1 x 3 square feet, it makes growth and working around the crop easier. Thus, we deciTesting for Minerals: What is ICP-MS did to divide the harvest area by 1/3 or 14, 520 feet of rows in the acreage and about 7, 260 in the ½ acres to make planting and working around the lettuce easier.

2. Thomasnet (2010) and Huang, (2016) shows that there are generally 20 - 24 heads per box in a corrugated box that is Size: 16"W x 24-1/4"L x 91/2"H, and they are priced at about 1-249 cases $2.18 per head to 1250 can be as low as $1.82 / head of lettuce.

Growing organic vs. synthetic

According to *Sakhi, Ms, D., Arabella, H., Ms, A., Aikenhead, E., Allen, K. and Lock, R. (2009)* there is a lot of research and consideration of organic vs. synthetic or organically vs. conventionally produced foods and their benefits and this is confirmed by a systematic review of literature by nutriwatch (2009). There are arguments for and against organic vs. synthetic growth processes and vegetables. The authors claim there are little differences in the nutrient content of the two growth processes for certain vitamins and minerals such as: vitamin C, calcium, potassium, phosphorus, soluble solids, copper, iron, manganese, sodium, plant carbohydrates and other minerals. They affirmed that in some cases there were significant differences in the minerals and element contents of the varied growth processes. In the major nutrients addressed, most of the organic crops had higher mineral contents.

In the systematic reviews, Paull, J., Kristiansen, P., & Hill, S. (2013) showed that organic farming has grown from 15.8 million hectares to over 37.2 million hectares globally in 10 years. Organic farming encompasses numerous restrictions on what can and cannot be used to classify gardens as organic but these also include a limited use of some synthetic fertilizers and chemicals. They show that India ranked seventh globally with 1.2 million hectares of approved and recognized organic agriculture or about 0.6% of its cultivable area.

Hollyer, J., Brooks, F., Fernandez-Salvador, L., Castro, L., Meyer, D., Radovich, T., (2013) showed that certain conventional fertilizers, pesticides, and synthetic or non-organic substances are used in U.S. farming. Under the USDA National Organic Program, Hollyer (2013) affirmed that certain synthetic, and conventional pesticides and fertilizers are allowed in gardens labeled as organic in the marketing strategies. There are numerous factors that govern the organic vs. synthetic label and consumers should be very diligent in assuming what they purchase and the real facts regarding the comparisons. The governing authorities have allowed non-synthetic and synthetic fertilizers to improve soil fertility, organic farming, and plant health. There are laws under Title 21 of the 1990 Farm Bill and Title 7 CFR Part 205 that govern this and the rules are different for conventional farmers vs. organic farmers. They are governed by several other laws as well: section 6502(21) of the Act (7 U.S.C. 6502(21)), the Rodenticide Act (7 U.S.C. 136(u) and National Organic Program CFR. Title 7: Agriculture, Part 205 (2).

According to Arizona State University (2013), common citizens consider organic foods as those are natural and prevention some illnesses, increase the quality of life, are organically grown foods vs conventionally grown foods. There are many misconceptions of classifying foods as organic vs synthetic and people need to make sure that the classifications are clear.

Some of these reasons include (1) few or no pesticides (2) organics are gentler (3) they believe organics have more nutrients. Thus, organically grown does not necessarily mean free of all toxins used in plant and animal production, according to Paull, J., Kristiansen, P., & Hill, S. (2013), there is much to understand about organic vs synthetic growing and one has to be very familiar with the production and growth process or grow it and control the process personally or know the people who are controlling it as in China, according to Paull, J. (2007 -2008).

Table 1: Organic vs. synthetic origin of growth

Organic Fertilizer	Synthetic Fertilizer
Animal manure	Aquatic plants (alkali extracted)
Blood meal	Boron
Bone (and meat) meal	Fish products (liquid, pH adjusted with acid)
Calcium chloride	Iron phosphate
Compost: animal manure or plant based	Iron sulfate
Fish meal/shrimp	Magnesium sulfate
Gypsum (mined)	Sulfurous acid
Guano (mined)	Newspaper
Feather meal	Micronutrients (such as zinc sulfate)
Decomposing crop residue	Lignin sulfate
Worm castings	
Seaweed	
Potassium magnesium sulfate, potassium sulfate	

Testing for minerals

In the article by USGS: Crustal Geophysics and Geochemistry Science Center (2013), Testing for Minerals: What is ICP-MS? And more importantly, what can it do? The Mineral content and primary manner of testing the effectiveness of this entire program and research was conducted with the United States Geological surveys “Inductively Coupled Plasma Mass Spectrometry or ICP-MS.” This is an analytical technique that has proven very effective for testing for elements or minerals. The United States Geological Survey (USGS 2013), the researcher tested for 25 minerals as follows. The minerals content for 25 were tracked: Al (Aluminum), As (Arsenic), Ca (Calcium), Cd (Cadmium), Co (Cobalt), Cr (Chromium), Cu (Copper), Fe (Iron), K (Potassium), Mg (Magnesium), Mn (Manganese), Mo (Molybdenum), Na (Sodium), Ni (Nickel), Pb (Lead), S (Sulfur), Se (Selenium), and Zn (Zinc) converted to parts per million (ppm) from mg, (ng/g), and other amounts. The previous researchers have shown that light enables the plants to successfully synthesize and store nutrients.

The purpose of this proposal was to research and test how to successfully grow two types of lettuce 1) Green salad bowl and 2) Gourmet lettuce under Supplemental lights vs. No supplemental lights with a high traceable mineral content in six weeks using hydroponic methods. It was to test the effectiveness of Supplemental lights vs. No Supplemental lights on quality, quantity, and effectiveness lettuce production. After six weeks of growth, the researcher proposed a mineral content type where the concentration of nitrogen shall be measured using the (ICP-MS). This tested the mineral content in 120 high mineral eatable lettuces and tested the mineral content with ICP-MS Machine and the ICPM Methodology. Thus, the Independent variable was the supplemental lights vs. no supplemental lights and the dependent variable was the lettuce with high mineral contents. The researcher proposed and showed that the two types of lettuce could be successfully grown hydroponically in a Colorado greenhouse and tested for minerals and this methodology could be generalized to other arid areas. Water and the vitamin content are not a part of this research. The lettuce was grown at room temperatures of 20º-22ºC a day and at night 15º-18ºC. Each type of lettuce has 60 samples – total of 120 samples in replicate two and replicate three is the same.

The ICP-MS technique was commercially introduced in 1983 by the UGGS and has gained general acceptance in laboratories. Geochemical analysis labs originally adopted the

ICPMS technology because of unmatched detection capabilities, especially rare-earth elements (REEs) and minerals – the subject of this study. The ICP-MS has numerous advantages over past techniques involving atomic absorption, optical emission spectrometry, and the ICP Atomic Emission Spectroscopy (ICP-AES) including:

1. Detection limits for minerals and elements equal to or better than those obtained by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS).

2. It has higher throughput than GFAAS machines and methods.

3. It gives one the ability to handle matrices with minimum interference.

4. The ICP source has a high-temperature source.

5. Superior detection capability to ICP-MS and ICP – AES with the same sample throughput.

6. Isotopic information output.

The ICP-MS machine was used to complete the detection of the minerals being tested. (See the list of 25 minerals). Since being commercialized for over 20 years ago, the ICP-MS has become a widely-used tool, for both routine analyses and for research in a variety of areas. The USGS affirms that it (ICP-MS) is a flexible technique with many advantages over traditional techniques for testing minerals. The ICP-MS also saves time, multi-elemental, Multi-mineral technique with much lower level capabilities. This machine was used to answer the key questions about the success of growing under the Supplemental lights and mineral retention. The specific purpose of this literature review was to critically examine the facts regarding the Evaluation of the Effectiveness of Supplemental lights vs. No supplemental lights or Regular Light upon lettuce and its Production and Retention of 25 minerals. This objective has been met and explained. These objectives were met in determining if lettuce can be successfully hydroponically grown in a Colorado and other greenhouse and that process can duplicate in arid climates to increase overall lettuce production using Supplemental lights vs. No Supplemental lights globally. The researcher tracked the production and retention of 25 minerals in the 76 heads of lettuce produced under these conditions. The researcher has also shown through this Literature Review that there is a critical need for more research in this are using Supplemental light vs. No Supplemental lights and fertilizer, the focus was not on the various colors of Supplemental lights, pH factors, and other variables that affect lettuce growth.

IV of lights vs. DV of lettuce

This review has examined and addressed and the DV of Lettuce and IV of Supplemental lights and it is positive or negative effect on lettuce growth in controlled growing lettuce hydroponically in greenhouses. The research has shown that a controlled Supplemental light environment, whether it is Red, Green, Blue, or White Supplemental lights has a more positive effect on the production of lettuce that No Supplemental light, Regular light, and/or uncontrollable arid Sunlight. We also examined the controlled environment of green houses and various researchers affirm that a controllable environment allowed large or small farmers and families to grow and produce quality lettuce on their own time schedule and limits. These findings can result in a positive impact on the manner in which persons in arid Midwestern and desert climates in the Middle East, Kuwait, Saudi Arabia, other cities, countries, and/or continents can improve their lettuce growth and food production. The researcher used the lettuce (*Lactuca sativa) or* green salad bowl and Gourmet blend mix to complete the study at Colorado State University's horticultural greenhouse environment. These findings will allow the researcher and others to use controlled inside environments, primarily horticultural greenhouses to grow lettuce globally at times and in places that were not possible before.

Results of the literature review

This literature review has successfully shown the need for Supplemental light vs. No supplemental light research and the critical need to additional research to fill the gaps of needed research and information to increase the production of lettuce in greenhouses hydroponically. It has shown the need for more improved methods for growing lettuce in hydroponically controlled greenhouses to increase the production in arid and desert areas where water is at a premium with long lasting Supplemental light s vs. Regular lights, Sunlight, or No supplemental light. Generalized to the broader populations and other crops, this research has shown that the researcher can have a significant impact upon the global production of food and the reduction of hunger and starvation in arid, wear torn, and other areas where temperatures reach extremes that make it difficult, hard, or almost impossible to grow food with ease. This literature review has shown that there is a critical need for more improved and a greater number of Hydroponic Supplemental light operated greenhouses for improving the production of lettuce in arid and desert areas of Colorado, the Middle East, Africa, and other areas. These literature review findings can be projected or generalized to the other parts of the world: American Deserts, Africa, the Middle East, Arabic Nations, Brazil, great deserts, and other dry climates on the globe.

CHAPTER 3: MATERIALS AND METHODS

The rationale of the study is to compare the effect of various amounts of Supplemental lights vs. No Supplemental lights on lettuce production and mineral retention. This research is a mineral content type of Organic vs. Synthetic hydroponic lettuce growing at CSU. Greenhouses and Supplemental lights vs. No supplemental lights with lettuce being the Independent Variable (IV, NCES, 2016, Footnote[1]) and Supplemental lights the Dependent Variable (DV, NCES, 2016, Footnote[2]). The aim of the study is to use the Independent variables of supplemental light s, no supplemental light and regular tap water to change the Organic lettuce and Synthetic hydroponic lettuce in the CSUFC. Greenhouse (NCES, 5/2016, p. 1[3]). The lights and water will be used to affect the lettuce's quality, quantity, mineral content, and other qualities of the fully developed lettuce. The researcher did three replicates of 120 plants [60 Organic and 60 synthetics], (R1, R2, and R3) will be used. In replicate 1 there were 60 organic and 60 synthetics (total 120 plants) however, in replicate number one No Supplemental light were used to this replicate 1. In replicate two, there were (60) organic and (60) synthetic for a total of 120 plants. In replicate three 3, (60) organic and (60) synthetic, total 120 plants. Replicate two and three were done at the same time and the researcher used Supplemental light vs. No Supplemental

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[1] An independent variable is exactly what it sounds like. It is a variable that stands alone and is not changed by the other variables you are trying to measure.

[2] A dependent variable is exactly what it sounds like. It is something that depends on other factors. For example, "The IV (Independent variable) causes a change in DV (Dependent Variable) and it isn't possible that (Dependent Variable) could cause a change in (Independent Variable). For example**:** (Amount of LED and REGULAR/NO LIGHTING) causes a change in (Lighting)." Note: IV and DV words change to fit this study rather than study /grade variables used at nces.ed.gov.

light. That is what my research is about, testing R2 and R3. The aim of the Supplemental will be to determine if the lighting will positively affect the growth and quality of the lettuces:

(1) Similar / improved quantities (yields) (2) Quality (nutrition, sensory), and (3) Is it worth the investment. The experimental day and night temperature will vary from 20º-22ºC and 15º-18ºC respectively or approximately 68 degrees Fahrenheit +/- five (5) degrees. Additional materials will be needed and explained later. We investigated and showed the critical need for the changes in methodologies, lighting, and other independent variables and how they affected the Dependent Variables. The researchers' review of literature will arrive at clear written conclusions about the body of peer-reviewed research, their finding, and valid conclusions over the last 10 years.

Timeline for the proposal

The Timeline for the research and proposal was for spring 2016 to summer 2017. This included writing the final draft of the proposal, setting up the study, gathering all the materials, preparing the greenhouse planting, harvesting, and freeze drying, testing, and examining the final composition of the lettuce.

Growing process

This research was conducted at the CSU P.E.R.C. Greenhouses complexes. The lettuce seeds were sown and grown in the Spring of 2016 in Rockwool from which were approximately 4x4 inches with a 1-2 cm x 1-2 cm hole and placed in the Hydroponic Tanks for maximum day temperature was 20ºC-22ºC and the minimum night temperature was 15ºC-18ºC inside the Greenhouse. Greenhouses Complexes. Generally, it took 14 days for the seedlings to grow successfully, 20 days, the grow cubes were placed into Rockwool grow blocks, 30 days for the lettuce to grow from seed to transplanting them to a permanent location. Growth was accelerated and the Lettuce had two months to grow to harvesting maturity, 2 weeks to seed and 6 weeks to grow to maturity. Data was collected and tested from July 2016 – November 2016. In (Lettuce quality, size, etc.) and it isn't possible that (Lettuce quality, size, etc.) could cause a change in (Lighting).” Note: IV and DV words change to fit this study rather than study /grade variables used at nces.ed.gov. (5/2016, what are Independent and Dependent Variables?”

Root zone treatments

The study compared the growth of lettuce in a Supplemental light vs. No Supplemental lights environment using hydroponic rockwoo1 culture. The dimensions of the hydroponic tanks and the rockwool are round planters. The dimensions of the entire bed were round and suitable. The Rockwool slab culture is commonly used in hydroponic perennial, vegetable, and food production (Succop, C., 1998). The experiment was set up in a Complete Round Blogged Design with treatments and replications with Hydroponic Organic Lettuce (*Lactuca sativa*).

The researcher used organic vs. synthetic fertilizer and kept checking for the EC every 2-3 days, measuring, and tracking of fertilizers in this study at Colorado State University in Fort Collins, Colorado (CSUFC). However, our purpose was to investigate the content of 25 minerals that are produced and retained in the Hydroponic growth process of Organic lettuce products. The goal was to determine the efficiency and success of CSU’s Hydroponic water growth methods, mineral retention, and final production. The greenhouse settings under Supplemental lights vs. No Supplemental lights or normal sunlight were a controlled environment for regulating all aspects of the research study. The general greenhouses of Colorado State University, Ft. Collins were the growth and experimental center for all this research, water, and light control studies. Both ends of the greenhouse are enclosed and the inside temperature is continually controlled and regulated. The hydroponic growing tanks replaced the typical earthen soil based growth methodologies in the greenhouses. The design of the greenhouse is as follows:

there were three rows for planting and two walkways about 2 feet wide. The entire greenhouse was approximately 70 feet long by twenty feet wide and about eight feet tall. There were two walkways between the three plots and the entire structure was covered with plastic. Each of the rows or tanks contained each tub transmits 30 plants (15 Green Salad Bowl) and (15 Gourmet) plants with a total of 360 with the R1, R2, and R3 tests, lettuce plants for this research. According to home.howstuffworks.com[3], the spacing of the lettuce plants.

Fertilizer treatments

There was regulation, measuring, or detailed tracking of fertilizers and the amounts of fertilizers used in this study. The tracking, use, and measurement of water and the vitamin content of lettuce are not a part of this research. Lettuce needs soil, fertilizer, and/or hydroponic care for proper growth to maturity and marketability. Leaf and Bibb lettuce are most acceptable but all types of lettuce will grow successfully in a hydroponically controlled environment. Lettuce feeds poorly and needs nutrients from the water, fertilizer, and soil and this research has shown that it is successful in either medium. However, organic fertilizer and synthetic fertilizer were used in this study as a DV.

Hydroponics irrigation system

The irrigation system used as the hydroponic methods, hydroponic technology, and Hydroponic rockwoo1 culture, all described as hydroponic methods in this study. The researcher did not use the traditional soil based methods for growing the lettuce or traditional /normal rainwater, tap water outside, peat moss, bag mix, perlite, Rockwool growing systems, organically fertilized plants and the salt-based fertilized plants. All for the irrigating was completed via the hydroponic methods.

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[3] http://home.howstuffworks.com/vegetable-spacing-guide.htm

Plot design and pest management

The CSUFC Plot design was the inside of the CSU. Greenhouses and included one main isle and two side rows for growing plants and separating them according to the protocols.

Figure 1: Rockwool in the CSU

Figure 2: Rockwool in the CSU

The plants were separated by planters and 120 plants were planted. They were constantly monitored and the Supplemental lights were regulated and timed according to protocols vs. No Supplemental lights. There was little need for pest management in such a controlled environment but if there was a need the pest controls it would be minimal since the area is enclosed. If there is a need for pest control, less than 5-10% of what is normally used outside under the open-air elements would be necessary. Thus, pest control is not a vital part or relevant in this study.

The pictures are the CSUFC Plot design inside of the CSU Greenhouses.

During this study, the researcher purchased materials for the study and used parts of the CSU Greenhouses. Prior to starting the research, the researcher shopped the following items were purchased for the experiment: (1) One 100' air hose, (2) Six 520 Gph Pumps, (3) two Power strips, (4) Styrofoam, (5) Net Pots, (6) Rockwool, (7) two types of fertilizers (organic and nonorganic), (8) two – Four Head Air Pumps and (9) stones. Then she purchased the two types of Lettuce seeds (Green Salad Bowl and Gourmet Blend Mix). After harvesting, the researcher has the lettuce stored in the freeze -20ºC and later freeze dried to be able to do the ICP-MS test. The Researcher will only examine samples from replicate two and replicate three. To save time and money only 40 random samples from R2 and 40 random samples from R3 were used.

The criteria for the fertilizers included that with 125 ppm of Nitrogen as follows: Organic: Age Old Grow (12-6-6) and the total amount used per tub was 588.15 ml. The secondary type was a synthetic (synthetic) fertilizer called Winfield (14-4-14) and the total amount used per tub was 4693 ml according to the directions of the past researchers and package. The temperature in the Greenhouses was regulated as follows:

1. 20º-22ºC or 68º – 71.6ºF in the day.
2. 15º-18ºC or 59º – 64.4ºF at night.
3. After harvesting they are stored in the freezer at -20ºC.

The researcher used the complete Round Blogged Design and randomly labeled the plants. The EC was taken every other day to check on the plants with organic tubs at 0.4 and synthetic tubs at 1.00. The seeds were stored in the freeze 20ºC and are later freeze dried for the ICP-MS test. A piece of Rockwool for each type of lettuce was used. The researcher placed them on a Mist Branch watered for 20 seconds every hour for two weeks in the germination room the temperature75ºF. The researcher then transferred the lettuce to the greenhouse room and had four tubes the size is 102.5* 42. The researcher added water up to eight (8) cm. The 102.5* 42 * 8 = 34,440, which equals to 149 gallons of water in each tub was used. Afterwards, two tubs where mixed with organic fertilizer and the other two with Synthetic Fertilizer. (Organic fertilizer is “Age Old Grow 12-66) (Synthetic Fertilizer is Winfield 14-4-4) and according to Cornell University needs to be 125 ppm, so the researcher did the calculation. Afterwards, the researcher placed the Styrofoam with 30 spots in each tub. Each tub had 15 plants labeled Type 1 and 15 plants were labeled Type 2. The replicate protocols were as follows: Replicate 1. For four weeks, long or a total of six weeks from germination. However, in replicate two and three the researcher did them at the same time to save time. The seeding included 3-5 seeds in each piece wool. There was a total of 30 Rockwool of each type of lettuce or 60 pieces of Rockwool total.

In the first round, which will not be examined, the lettuce was grown as follows: there were 60 Green and 60 Gourmet, but 30 greens were grown in organic fertilizer and the other 30 green were grown in the synthetic fertilizer. The same was done in the Gourmet Blend.

The following products or tools were purchased: Two lettuce types for two treatments (one organic, one synthetic), Two types of lighting One (1) Supplemental vs. (2) No Supplemental, for Three (3) replicates that totaled 240 plants. Rounds two and three of the growing process were done simultaneously for a total of 240 plants with the same structure of round one. However, the researcher added Supplemental light to ½ or 60 plants and No Supplemental light to the other ½ or 60 plants. In round two and three there were 4 tubs. Four tubs were under the Supplemental lights (2 organic and 2 Synthetic) the other 4 tubs no supplemental lights (2 organic and 2 Synthetic). The EC was taking mostly 0.3-0.4 for the organic tubes. EC for the Synthetic was between 1.0 - 1.2.

The researcher placed them on a mist branch watered for 20 sec. every hour for two weeks in the germination room the temperature 75ºF. The researcher then transferred the lettuce to the green houseroom and had four tubes the size of 102.5* 42.

The researcher used four samples of lettuce which took 7-9 days to freeze dry: four lettuce samples took 7-9 days using the Freeze dry protocol step by step for each of the two sets or a total of 80 samples. The Mill Equipment is used for Grinding in Agricultural Research Development and Education (ARDEC). This process is used for the 80 samples/day at 15 seconds per sample. Afterwards, the researcher started the Digestion with Dr. Chaparro using the following steps and the ICP-MS test to examine some elements of the lettuce and the 25 elements. This confirms that this thesis is a mineral content type of 1) organic vs. Synthetic hydroponic lettuce in a green house and 2) Supplemental lights vs. No supplemental lights or Regular Light.

Freeze drying protocol

The researcher used every four samples of lettuce in 7-9 days using the Freeze Dry Protocol step by step for a total of 80 samples. The grinding equipment name is (Mill) and is used in Agricultural Research Development and Education (ARDEC). This process was used for the 80 samples a day and 15 seconds per sample. The researcher then started the Digestion under the supervision of her professor Dr. Chaparro for the mineral content type of 1) Organic vs Synthetic Hydroponic lettuce in the Greenhouse and 2) Supplemental lights vs. No Supplemental lights.

The researcher started grinding Acid Digestion and concentrations and then she weighed out samples of the digested samples to create a standard curve. Afterwards the samples were tested on ICP-MS and worked on analyzing the data from the research experiment as follows.

This VirTis 25LL. Freeze Drying Protocol was drafted by Shawna Matthews on about 12/20/2013 and must be followed exactly according to the Shawna.

Digestion methodology

The digestion Methodology is the process whereby the Researcher used the ICP-MS to determine the sub-µg/L concentrations of the 25 desired minerals or elements in the lettuce via water samples, waste extracts, or digestion. The constituents were filtered and the acid was then preserved before analysis. According to Environment Protection Agency (EPA 2015), the digestion of the elements is not required if dissolved in water. Some Acid Digestion is required for certain elements such as groundwater, industrial wastes, sludges, soils, sludges, aqueous samples, and other solids before filtration and analysis. The data determines method of analyses. This digestion methodology can be used to test for more than 60 elements but we were testing for only 25 (www.epa.gov, 2015). Sometimes multi-laboratory testing is necessary but not in this research.

According to quimlab.com.br (2017), the digestion analysis methods used to determine metals and minerals by the ICP – MS for quantitative recovery of metals. Data from the digestion methods can be discovered and tabulated into tables and charts. According to agilent.com (2009) and caslab.com (1994) the digestive process can be used for the quantification of cadmium, lead, chromium, mercury and other minerals and metals using the ICP-MS system. It can also be used for Isotope dilution analysis as a confirmatory technique. In this work, it is a highly accurate and precise method. Below are the steps of ICP-MS. After the researcher started the processes (Grinding: Acid Digestion and Concentrations), the samples were weighed then digested the samples to create the standard curve and ran the samples in/on the ICP-MS and analyzed the data as follows. The data analysis must be followed exactly according to the Author: Jacqueline Chaparro in SOP: SOP019, created: 09/07/2016 and later updated. The Title is: SOP for ICP-MS Sample Digestion and the methodology is as follows with all credits to the author and unaltered and not plagiarized because it cannot be altered or paraphrased: See the protocol entitled: SOP:

SOP019, created: 09/07/2016, updated: Author: Jacqueline Chaparro, SOP FOR ICP-MS Sample Digestion, Materials and Reagents ([i]) for the exact steps used in the process.

CHAPTER 4: RESULTS AND DISCUSSION

Introduction to results and discussion

The lettuce in this study was planted and harvested approximately six weeks after it was planted and then it was analyzed. The researcher proposed and examined the key literature and research from major food, agricultural, and horticultural theorist and grew the lettuce in a controlled agriculture and horticulture environments. The researcher then used a mineral content type Induced Coupled Plasma Mass Spectrometry (ICP-MS). This tested the mineral content in 76 heads of lettuce and the mineral content via the ICP-MS Machine and the ICPM Methodology. Thus, the Independent variable (IV) was the Supplemental lights vs. No Supplemental lights and the Dependent Variable (DV) was the lettuce with high mineral contents. The researcher showed that the two types of lettuce could be successfully grown hydroponically in a Colorado greenhouse and tested for minerals. Water and the vitamin content were not a part of this research but the fertilizer effect could not be ignored in the final analysis vs. the Null Hypothesis of Supplemental lights vs. No Supplemental lights and its effect on the mineral content of e lettuce.

The Researcher used the ICP – MS analysis of variance with a 2-Way or 3-Way ANOVA for each of the 25 elements we were testing for in the ICP-MS for testing the elements. The samples tested were cultivar, fertilizer, and lights. The test measured statistical validity and reliability of the findings. The researcher also used the assumption of homogeneity of variance (AOH) where all comparison groups have the same variance. The independent samples t-test and ANOVA utilized the t and F statistics respectively. Afterwards, the researcher ran the statistical and mineral tests for 25 elements via a 2-way and 3-way ANOVA and found that all interactions were not significant; there was not a difference sufficient to prove validity and reliability of the Supplemental lights effects on the minerals vs. No Supplemental lights. The test did not violate the assumption in that the group sizes were equal but failure to reach a P value of 0.95% did cause the null hypothesis to be rejected. However, the research findings and study were not useless.

Raw data analysis

The experimental methodology used in this study included freeze drying the lettuce leaves (Gourmet or Green) and testing the effects of Organic and Synthetic Fertilizer on that lettuce where Supplemental lights vs. No Supplemental lights was used as the IV and DV in two experiments (see the methods section). Approximately 100mg of ICP MS freeze dried lettuce was placed in 13x100mm culture tube to make it an evaluable concentration based on the Limit of Blank (LoB), Limit of Quantitation (LoQ), and Limit of Detection (LoD) and the explanation of that is below. This made 1.5 mL of 70% usable and properly concentrated nitric acid analyte solution (BDH Aristar® Plus) that was added to the Freeze-Dried Lettuce (FDL). This analyte concentrated solution was added and followed by 66.7 µL of standard analyte solution (internal) whose concentration is 10 ppm for each of the five elements (Sc, Ga, Y, In, and Bi). Then the lettuce samples were evenly mixed, covered up with plastic, and the digestion process took place overnight at the unmodified room temperature unless it was too hot or cold for the research protocols.

Later the samples were mildly heated in a sand bath (heating bath) 180 minutes or 3 hours at 120°C or 248^{0} F. After the 180 minutes or 3 hours, the tested samples were removed from the heating or sand bath and cooled for 5 minutes. Subsequently, the 750 µL of hydrogen peroxide (J.T. Baker, 30% Ultrex® II Ultrapure reagent) concentration was added to each sample to secure the proper concentration and the solution was reheated in the bath for 60 minutes at 120°C. The freeze-dried lettuce samples were then removed from the heating sand bath and cooled to the protocols room temperature. The modified solution was then transferred to a 15mL centrifuge[4] tube and pure water was added to get it to the proper concentration of diluted to 10-mL. Afterwards the 4.5 mL of modified solution was then moved to another 15-mL centrifuge tube for testing. Subsequently, pure water was added to the solution to dilute it to 15-mL which changed the internal standard concentration from more than 20 ppb to an even 20 ppb in three percent (3%) nitric acid. Thus, the researcher will present the significant main effect of the study which is of great value, there is still global need to improve the growth and production of lettuce under controlled Supplemental, Non-Supplemental lights conditions in Horticultural rich greenhouse conditions. Such controlled environments are needed in all arid countries in the world where there is a shortage of more than 20 – 30 million tons of food for the overall populations involved and the import and export practices do not always meet the needs of the population.

The researcher will use the Internal Standard as part of the analysis. The Internal Standard is a compound difference between "internal standardization" and "external standardization" is the former works with valid area “ratios” and accepted concentration ratios rather than “areas and concentrations.” Computationally, "internal standardization" works best in the statistical analysis in some cases (2017, medical-dictionary.thefreedictionary.com[5]). Likewise, the substance being analyzed is an Analyte (2017, collinsdictionary.com[6]). Internal standardization generally improves precision when the strongest or dominant sources of error in the family of or related to the sample preparation or injection. Likewise, thus, such errors affect

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[4] Definition: internal standard, LC Resources: http://www.lcresources.com/resources/TSWiz/hs330.htm

[5] http://medical-dictionary.thefreedictionary.com/internal+standard,

[6] https://www.collinsdictionary.com/dictionary/english/analyte, Collins English Dictionary, 2017

the internal standard and the analyte peak equally. Generally, they cancel out during the aerial calculations. The Internal standardization sometimes degrades precision when the primary sources of error are related to integration, separation, and/or peak shape because these errors affect the analyte[7] (substance being analyzed) and/or the internal standard peak differently, they generally with the calculation of the ratio of areas.

A 2-way interaction is defined a "To say that there is an interaction between the two variables means that the effect of one IV on the dependent variable (DV) depends on the level of the other IV ([8]). A 3-way interaction is defined by stats.idre.ucla.edu, 2017.as a situationwhere: "there is a two-way interaction that varies across levels of a third variable. Say, for example, that a b*c interaction differs across various levels of factor a (stats.idre.ucla.edu, 2017,[9])." The researcher also used the assumption of homogeneity of variance (AOH) for the error it was satisfied. According to statisticssolutions.com (2017[10]), the definition of the assumption of homogeneity of variance is:

"An assumption of the independent samples t-test and ANOVA stating that all comparison groups have the same variance. The independent samples t-test and ANOVA utilize the t and F statistics respectively, which are generally robust to violations of the assumption as long as group sizes are equal. Equal group sizes may be defined by the ratio of the largest to smallest group being less than 1.5. If group sizes are vastly unequal and homogeneity of variance is violated, then the F statistic will be biased when large sample variances are associated with small group sizes. When this occurs, the significance level will be underestimated, which can cause the null hypothesis to be falsely rejected. On the other hand, the F statistic will be biased in the opposite direction if large variances are associated with large group sizes. This would mean that the significancelevel will be overestimated. This does not cause the same problems as falsely rejecting the null hypothesis; however, it can cause a decrease in the power of the test."

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7 https://www.collinsdictionary.com/dictionary/english/analyte: Analyte noun, chemistry, a substance or sample being analyzed Collins English Dictionary. Copyright © HarperCollins Publishers P. 39

8 https://depts.washington.edu/psych/files/writing_center/interactions.pdf

9 http://stats.idre.ucla.edu/spss/faq/how-can-i-explain-a-three-way-interaction-in-anova-2/

10 http://www.statisticssolutions.com/the-assumption-of-homogeneity-of-variance/

The researcher satisfied the Normality of Error (NoE), which is defined as the "normal distribution theoretical frequency distribution for a random variable, characterized by a bell-shaped curve symmetrical about its mean, also called *Gaussian distribution (Normal* Distribution*) … (thefreedictionary.com 2017).”* Afterwards, the researcher ran the statistical and mineral tests for 25 elements via a 2-way and 3-way ANOVA and found that some interactions were not significant; there was not a difference sufficient to prove validity and reliability of the Supplemental lights effects on the minerals vs. No Supplemental lights. This will be further explained later.

However, the research, data, tests, and statistical analysis was far from useless and adds to the body of literature and research for the growth and production of lettuce in agricultural and horticultural situations and Supplemental light vs. No Supplemental light. Thus, the researcher will focus on and present the significance of the statistical data and the main effect of the study, which is of great value. There is still a global need to improve the growth and production of lettuce under controlled Supplemental, non-Supplemental lights conditions in agricultural and horticultural rich greenhouse conditions. Such controlled environments are needed in all arid countries in the world where there is a shortage of more than 20 – 30 million tons of food for the overall populations involved and the import and export practices do not always meet the needs of the population.

Statistical mode ([11]) tells us about the data point that is most frequently repeated in the dataset and if it is a symmetric data distribution, generally the statistical mode can be near the middle (mean and median). However, that can change for highly skewed data; the mode can be quite different or distinct. The Limit of Blank (a), Limit of Detection (b), and Limit of

[11] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2556583/

Quantitation (c) are terms used to describe the smallest concentration of a measure and that can be reliably measured by an analytical procedure.

1. LoB is the acronym for the highest *apparent* analyte concentration.

2. LoD is the acronym for the lowest analyte concentration. The formula is LoD = LoB + 1.645(SD low concentration sample)

3. LoQ is the acronym for the lowest concentration the analyte can be reliably detected when predefined bias and imprecision goals are met. The

LoQ ≤ LoD or it could be at a much higher concentration.

This presentation will use Flue Gas Desulfurization (FGD) waters and the ICPMS with Dynamic Reaction Cell (DRC) technology, which is optimized and validated to measure the quantification of minerals[12]. The Dynamic reaction cell is an apparatus that analyzes and detects gasses. The values of RPq and RPa control the mass band pass in the DRC. RPq is the low mass cutoff and controls the RF applied to the quadrupole rods in the DRC ([13]).

RPa is the high mass cutoff point, RPq is the low mass cutoff, and control the mass band pass in the DRC. These values control the mass band pass in the DRC.

RPq + low mass cutoff and directly affects the RF in the DRC (2015, pubs.usgs.gov)[14]. According to the statistics from the Ionomics analysis using ICP-MS (Chaparro, Jacqueline, 2016-11-07) Re Greenhouse Lettuce grown hydroponically where Ionomics analysis were applied and the outcomes showed that fertilizer used significantly affected the lettuce ionone and

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[12] Ag, As, Cr, Cu, Ni, Sb, Se, V, and Zn

[13] https://pubs.usgs.gov/of/2015/1010/pdf/ofr2015-1010.pdfP.41

[14] https://pubs.usgs.gov/of/2015/1010/pdf/ofr2015-1010.pdf P.41

minerals. The freeze-dried lettuce leaf was used for gourmet or green lettuce and the fertilizer was organic or synthetic in the test of Supplemental lights vs. no Supplemental lights for 1 or 2.

ICP-MS raw data analysis

During the ICP – MS Analysis, the elemental concentrations of 25 elements (Se, As, Na, P, Li, Be, B, Cd, S, Mg, K, Ca, Fe, Co, Ba, W, Ni, Cu, Al, V, Cr, Mn, Zn, Sr, Mo, and Pb) were measured. The instrument used was an Elan Dynamic Reaction Cell (DRC) II Mass Spectrometer ([15]). This was attached to a Quartz Cyclonic Spray Chamber and a Seaspray™ MEINHARD Nebulizer. The ASX520 auto sampler (CETAC Technologies) was used to introduce Samples. Eleven mineral or elements ([16]) were measured in the standard statistical mode. Oxygen was used as the reactive gas to measure three elements or minerals ([17]) in DRC mode. Ammonia was used as the reactive gas to measure 12 elements or minerals ([18]) in DRC mode. The Lens voltage were optimized and set for the maximum Indium signal intensity (ISI, 56008 counts per second), before analysis via the nebulizer gas flow and achieved 0.85 and 8.0 respectively. The outcomes of CeO+: Ce+ of 0.028 and a Ba++:Ba of 0.017 was achieved via a daily performance check which ensured proper instrumentation functioning and operation. By analyzing seven (7) dilutions the multi-element stock solution, which was made from mixing single-element stock standards (Inorganic Ventures), the proper calibration curve was secured. The pooled lettuce sample which was prepared by mixing 2mL of each of the digested individual samples was run every 10th sample and served to correct for instrumentation drift and flaws for quality control (QC) of the solution.

[15] PerkinElmer

[16] Li, Be, B, Na, P, S, Mg, K, Ca, W, and Pb

[17] Cd, Se, and As

[18] Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Mo, and Ba

The SAS system

In the General Linear Models (GLM) procedure, the researcher used the class level information of class, levels, and values. The classes were lettuce type (two levels of gourmet and green), fertilizer: (organic and two types synthetic), and light – (two values of Supplemental light and No Supplemental light). The number of observations read and the number of observations used were equal at 76 for each one.1

Data analysis and statistics

The research Data was compiled and processed using Excel and later SPSS and other statistical programs. Each of the 25 elements were subjected to general internal standard corrections and improved by the subsequently drift corrections [1]. For the QC samples, the statistical corrections were chosen and made based on process of minimizing the coefficient of variance (CV). After the drift corrections were made, all samples were modified and corrected for the dilution factor. To render the concentrations usable, the Limits of detection (LOD) and/or Limits of Quantification (LOQ) were properly calculated at 3X's or 10X'S the regular blank standard deviation divided by the slope, whether ascending or descending, of the respective calibration curve [2, 3]. The final concentrations are presented and analyzed in parts per billion (ppb, µg/L). The measured statistical calculations below the Limits of Quantification were paired or designated to the LOQ value for each element. The AOV or Analysis of Variance was calculated for each element via the function in R. The p-values were properly adjusted to eliminate false positives utilizing the Bonferroni-Hochberg Methodology in the p.adjust statistical function method in R [4]. PCA was properly conducted on UVscaled data utilizing the accepted PCA Methods package in the statistical R.

Statistical analysis & results

To analyze the effect of fertilizer, lettuce type, and light effect on 25 measured elements from the raw data (Appendix (A)), we consider three models. The first model was 3-way ANOVA for each element. Running 25 ANOVA in an experiment increased the experiment-wise error rate (EER or type I error). To control type one error, we consider grouping these 25 elements into two groups by using Principal Component Analysis (PCA). But, because of the overlapping of the important of some of the elements to the both first principal components (2 groups), we consider the Factor Analysis (FA) to eliminate or reduce the overlapping of the importance of these elements to the both groups by using the FA-Principal Component Method with Varimax Rotation.

Table 2: Summary of raw data

Summary of elements analyzed

Element	Abbreviation	m/z	Category	Mode	Reaction	Flow	Rpa	RPq
Lithium	Li	7	Analyte	Standard	None	-	0	0.25
Beryllim	Be	9	Analyte	Standard	None	-	0	0.25
Scandium	Sc	45	Internal Standard	Standard	None	-	0	0.25
Cadmium	Cd	110	Analyte	DRC	O_2	1.6	0	0.7
Selenium	Se	78	Analyte	DRC	O_2	1.6	0	0.7
Indium	In	115	Internal Standard	DRC	O_2	1.6	0	0.75
Sodium	Na	23	Analyte	Standard	None	-	0.12	0.7
Phosphorous	P	31	Analyte	Standard	None	-	0	0.7
Sulfur	S	34	Analyte	Standard	None	-	0.1	0.7
Magnesium	Mg	26	Analyte	Standard	None	-	0.11	0.7
Potassium	K	39	Analyte	Standard	None	-	0.12	0.7
Calcium	Ca	43	Analyte	Standard	None	-	0.11	0.7
Aluminum	Al	27	Analyte	DRC	NH_3	0.7	0	0.75
Vanadium	V	51	Analyte	DRC	NH_3	0.7	0	0.75
Chromium	Cr	52	Analyte	DRC	NH_3	0.7	0	0.75
Manganese	Mn	55	Analyte	DRC	NH_3	0.7	0	0.75
Iron	Fe	56	Analyte	DRC	NH_3	0.7	0	0.75
Cobalt	Co	59	Analyte	DRC	NH_3	0.7	0	0.75
Nickel	Ni	60	Analyte	DRC	NH_3	0.7	0	0.75
Copper	Cu	63	Analyte	DRC	NH_3	0.7	0	0.75
Zinc	Zn	66	Analyte	DRC	NH_3	0.7	0	0.75
Gallium	Ga	71	Internal Standard	DRC	NH_3	0.7	0	0.75
Strontium	Sr	88	Analyte	DRC	NH_3	0.7	0	0.75
Molybdenum	Mo	98	Analyte	DRC	NH_3	0.7	0	0.75
Barium	Ba	137	Analyte	DRC	NH_3	0.7	0	0.75
Tungsten	W	182	Analyte	Standard	None	-	0	0.25
Lead	Pb	208	Analyte	Standard	None	-	0	0.25
Bismuth	Bi	209	Internal Standard	Standard	None	-	0	0.25
Yttrium	Y	89	Internal Standard	Standard	None	-	0	0.25
Arsenic	As	75	Analyte	Standard	None	-	0	0.25

Table 3: Results Limits of Detection (LOD) and Limits of Quantification (LOQ) calculated to the undigested lettuce to measure and analyze element of the grown products.

	LOD ppb	LOQ ppb
Li	12.86	42.85
Be	46.93	156.44
Cd	7.33	24.43
Se	569.76	1899.19
Na	88135.56	293785.20
P	1474.60	4915.35
S	129928.70	433095.66
Mg	4341.18	14470.59
K	8367.16	27890.52
Ca	8189.89	27299.64
Al	5911.03	19703.44
V	219.78	732.61
Cr	11.25	37.49
Mn	1.85	6.18
Fe	156.75	522.49
Co	1.95	6.50
Ni	0.55	1.83
Cu	5.73	19.10
Zn	48.46	161.53
Sr	2.64	8.79
Mo	6.99	23.30
Ba	10.37	34.56
W	1.24	4.12
Pb	1.77	5.89
As	2.91	9.70

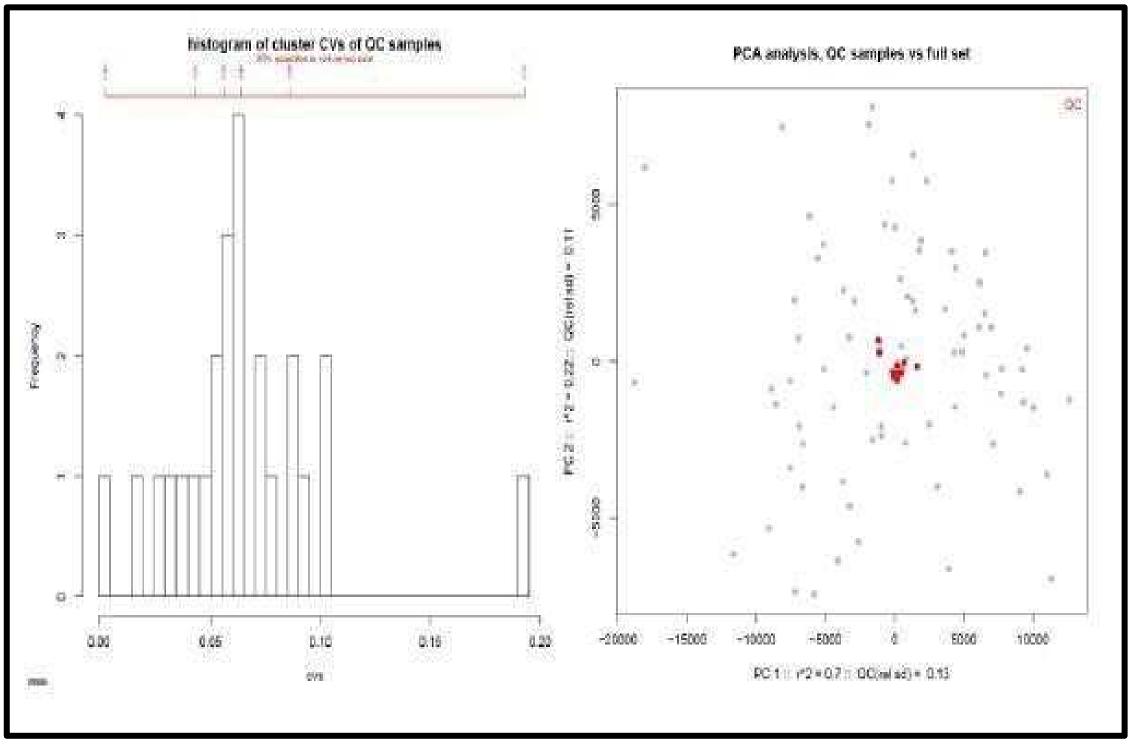


Figure 3: Quality control

The Quality control samples of the lettuce, which included pooled digested lettuce samples (pooling of 2mL of each of the digested individual samples) was run on every 10th sample of lettuce. The histogram Chart or diagram below shows that 80% of the QC samples had a statistical COV below 8.6%. In the unknown samples, the PCA analysis to the right shows that all QC concentration samples clung together and the analytical variance was small or very little.

Table 4:The CV outcome for each tested element across the accepted and used quality control samples

	CV
Li	2.64
Be	7.92
Cd	10.19
Se	19.30
Na	6.26
P	5.66
S	5.24
Mg	4.95
K	6.32
Ca	6.15
Al	9.04
V	3.46
Cr	8.95
Mn	6.46
Fe	5.11
Co	10.13
Ni	8.55
Cu	5.98
Zn	1.77
Sr	4.42
Mo	7.17
Ba	7.40
W	4.00
Pb	0.30
As	5.62

Table 4 shows the standard curve calculated of the individual elements with the statistical R squared value. These values show there are strong linear relationships between counts and the selected known element concentrations. The mathematical or statistical relationship between counts and standard concentrations at ppb are displayed or represented by the slope of the line and y-intercept point.

Table 5: Standard curve for R squared, slope, and y – intercept

	R squared	Slope	y-intercept
Li	0.997	189.038	399.642
Be	0.997	36.545	12.050
Cd	1.000	208.707	37.823
Se	0.986	10.773	6.957
Na	0.988	0.066	302.812
P	1.000	295.182	-307776.602
S	0.996	1.619	78287.240
Mg	0.996	0.797	12787.848
K	0.997	0.970	-15471.776
Ca	1.000	0.185	32.143
Al	0.996	20.306	197126.012
V	1.000	70.329	983.580
Cr	1.000	1081.687	-4575.307
Mn	0.997	3156.483	-255574.078
Fe	1.000	1942.664	-58027.793
Co	0.999	2706.137	-80.194
Ni	1.000	513.103	-86.910
Cu	0.997	1077.946	-3779.781
Zn	0.996	84.777	3895.425
Sr	0.999	11094.182	32090.786
Mo	0.996	377.409	-374.151
Ba	1.000	1447.269	-2299.664
W	1.000	4447.757	45.848
Pb	1.000	10816.813	-170.044
As	1.000	2294.187	177.509

PCA History: PCA is an unbiased statistical, multivariate analysis that explains percent variation from multiple variables in a single component or output. Each variable (compound) loading score and each sample component score, are plotted 2-3 at a time and compared vs. each other ([19]). PCA is generally used and gives a picture of or overview of multifaceted and multidimensional data. The coordinate (placement) of each sample (plot below) are plotted,

[19] e.g. PC1 vs. PC2, or PC1 vs. PC2 vs. PC3, or PC1 vs. PC4, etc.

placed, and defined by the complete profile of elements that were detected by ICP-MS and the protocol for statistically plotting it correctly. The more similar particles and ionomes elements cluster together, and the less similar or different ionomes are forced or pushed apart. A PCA biplot is numerically directional in two separate directions: left vs. right (plot PC1), and up/down for plot PC2. The right panel that is depicted below shows the collection or loadings of those PCs. The plotted loadings can be explained in the same directional placement as the PCs: if it is towards the upper right of the collection plot causes the separation of the samples and results in forcing the samples upwards in the upper right of the scored plots.

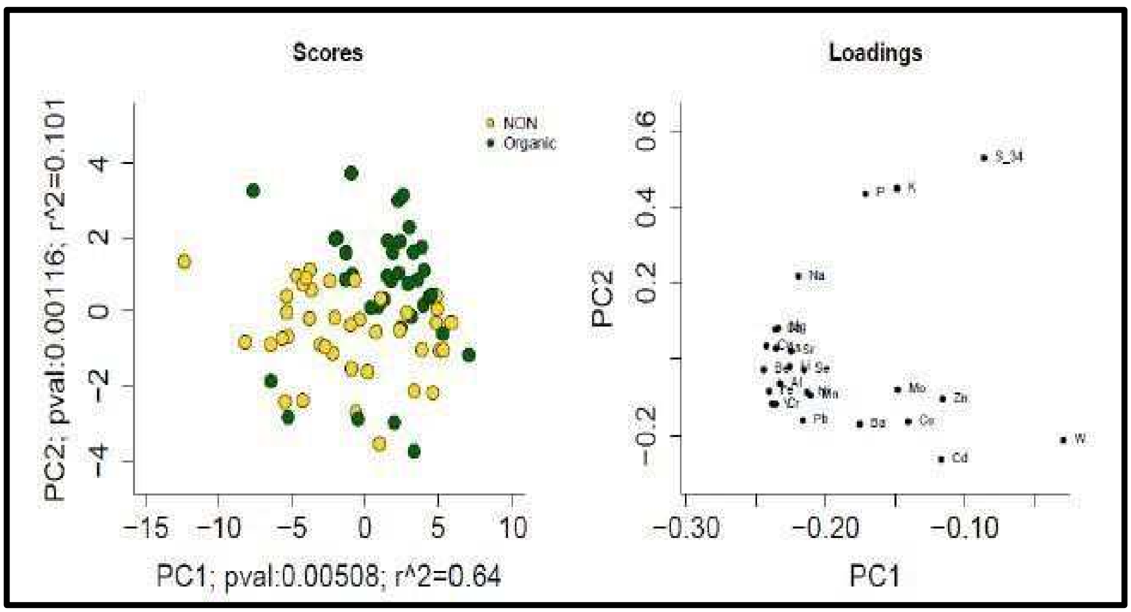


Figure 4: Pdata pre-processing: data was UV scaled ICP-MS data analysis - PCA

The Plots of PC1 and PC2 give data overview and can also unmask potential or real outliers. See the plots below where the scores are on the left, generally, and loadings plots are generally on the right. In this case, the complete variance described using PCs 1 and 2 is almost 74.1% of the complete dataset wide statistical variance and it shows a strong upper to lower separation by fertilizer influences. The collected and placed plot to the right plots the weight of every micronutrient or macronutrient and it contributes to the division observed on the separated scores plot. These data can be read or explained by mentally overlaying the pictures of the two plotted and colored plots. This placement of the plots and pictures show that P, K, and S help to separate differing samples along the final PC2 picture (y-axis), and Al, Se, or Zn helps divide the data along the plotted y-axis. Overall, these data recommend or point to large differences among the micronutrient and macronutrient content of lettuce (organic) and Nonfertilized lettuce samples that were evaluated.

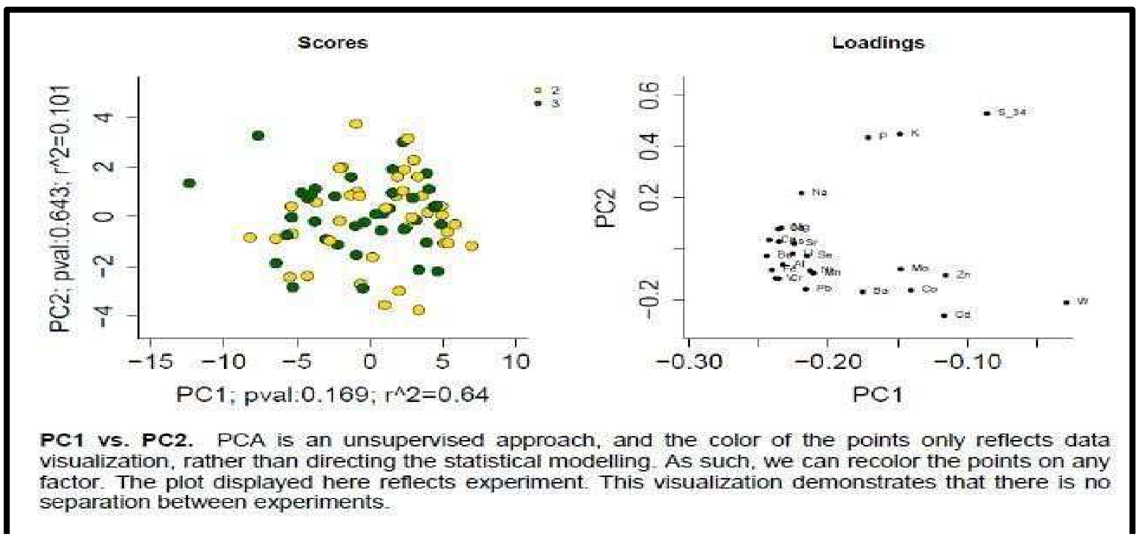


Figure 5: PC1 vs. PC2

Figure 5 an unmanaged and unsupervised approach were the dotted circle points reflects clear data visualization and does not serve to direct or influence the statistical modeling. Consequently, the colors can be changed for any plot and reference point as long as it is done throughout. The plot pictured here is a representation of the experiment. This picture visually shows there is no real separation between experiments conducted by the researcher, advisors, and/or assistants in the lab.

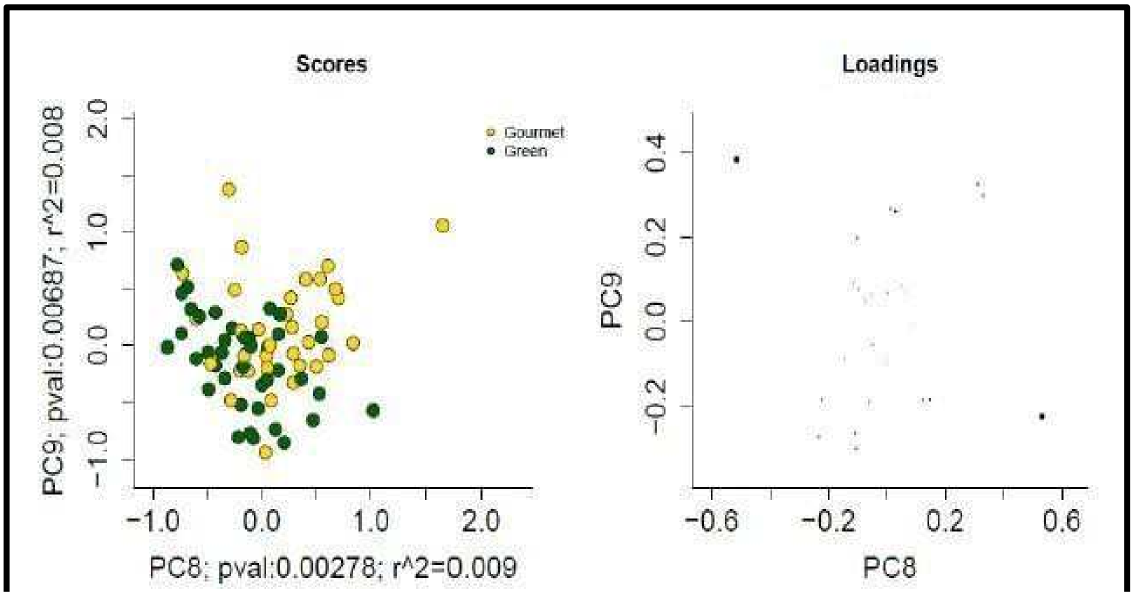


Figure 6: PC8 vs. PC9

The Principle component calculated scores are mathematical quantitative summaries of the complete metabolome. Since they are statistically quantitative and ongoing or continuous data we can successfully perform statistics on them with meaningful outcomes. The researcher's application of ANOVA to all of the PC scores show that PC8 and PC9 have a response to the category of each lettuce type. When the researcher plotted the PCs, which shows, that approximately 1.7% of the dataset wide variance and a minute percentage of the variance separates the two-lettuce type.

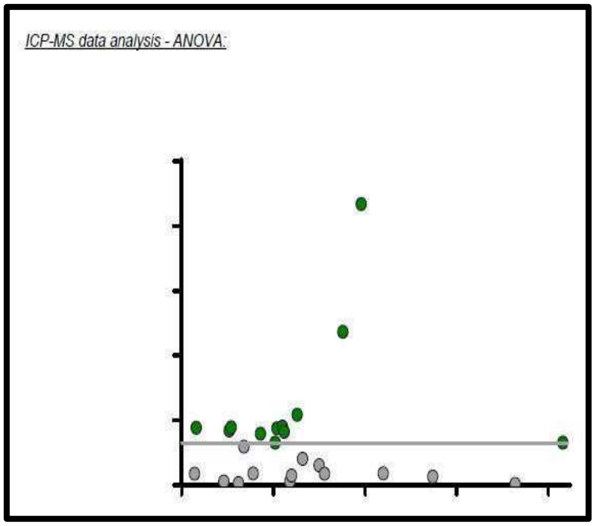


Figure 7: ICP-MS data analysis – ANOVA

Statistically, the ANOVA models were applied to all the micronutrients and macronutrients in the dataset of information. The statistical or plotted model consisted of Lettuce + Organic + LED + Experiment which means the main effects of the IV and DV's of Lettuce, Organic Fertilizer, Supplemental lights, and Experiment are tested or calculated and presented. The results of the calculations are plotted as a picture to present the function of molecular weight of the elements and the statistical –log off the P-value validity and reliabilities. The higher the numbers on the plot, the lower the p-value is statistically. All valid elements were colored as green fill or dots and represent statistically significant results of the calculations. The Calculated ANOVA results show that a statistically significant outcome to fertilizer – 12 out of 25 elements resulted and tracked a demonstrated BH corrected p-value of less than 0.05 – which is statistically significant and insignificant for the other for about 50% of the dataset.

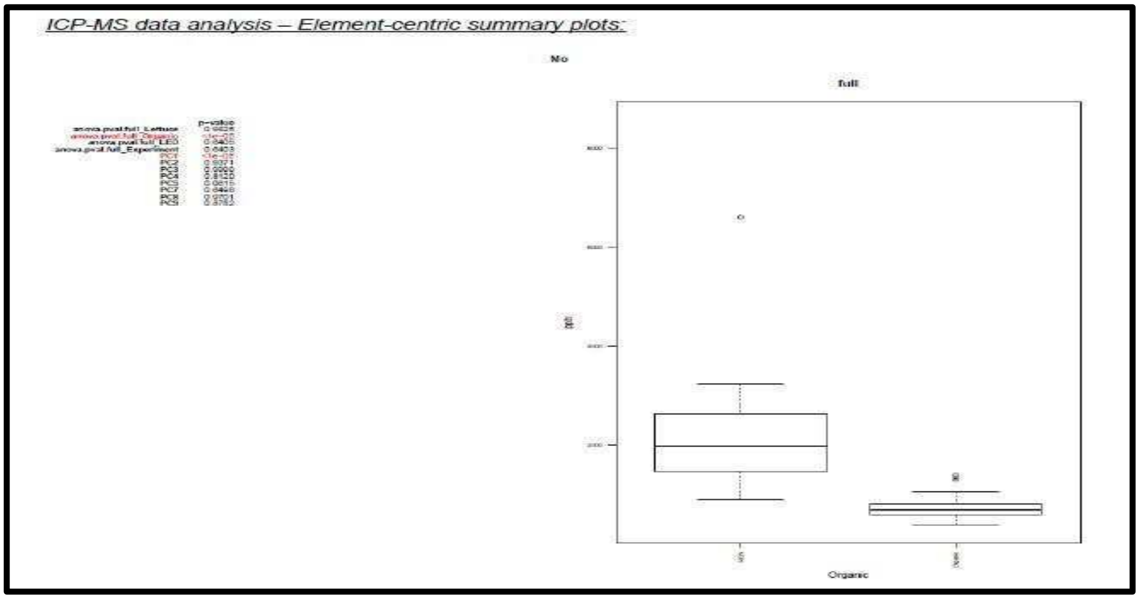


Figure 8: ICP-MS data analysis – element-centric summary plots

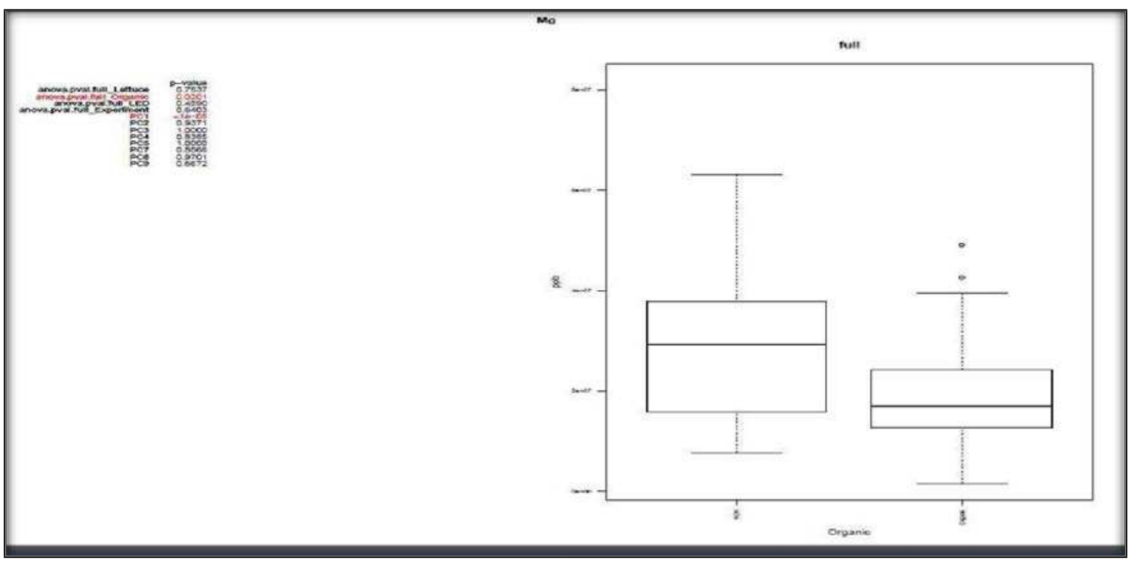


Figure 9: ICP-MS data analysis – Element-centric summary plots

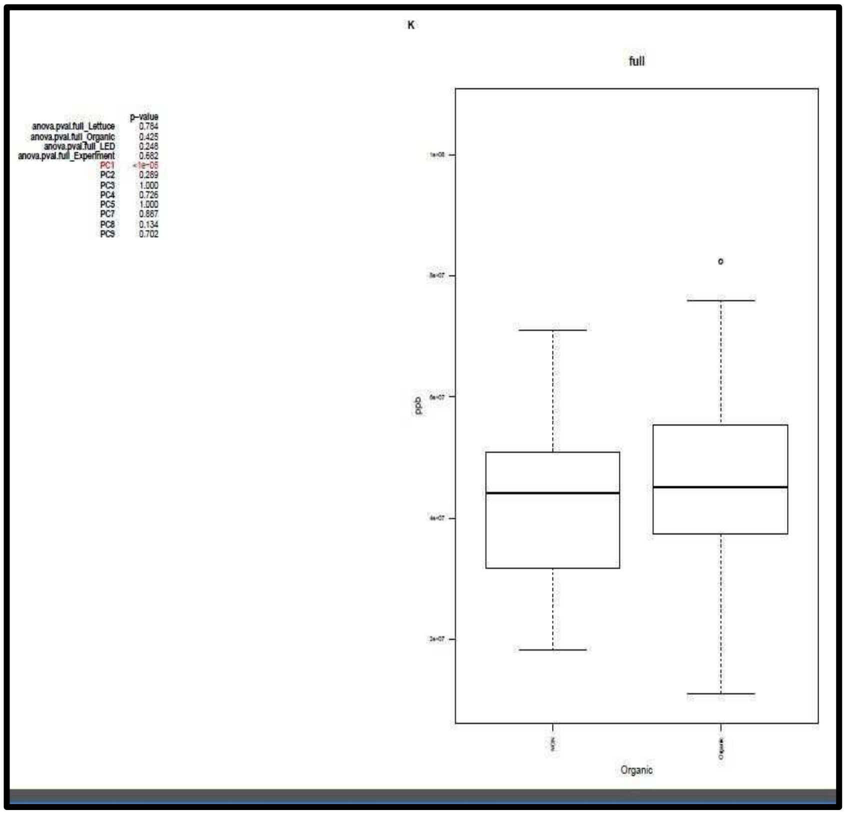


Figure 10: ICP-MS ANOVAs of K

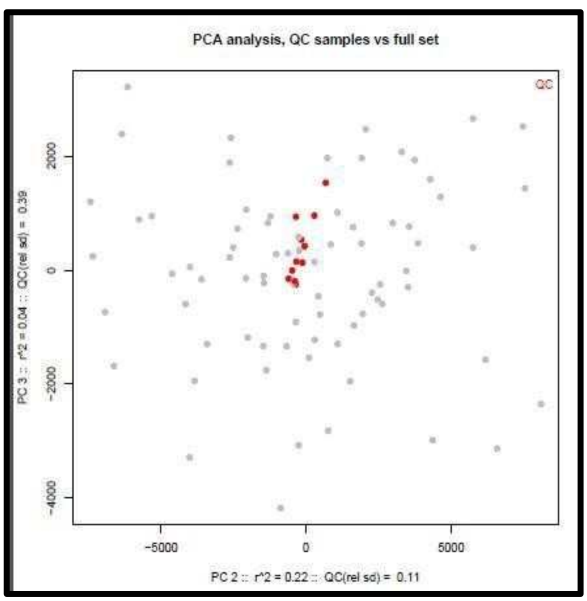


Figure 11: PCA analysis, QC samples vs. full set

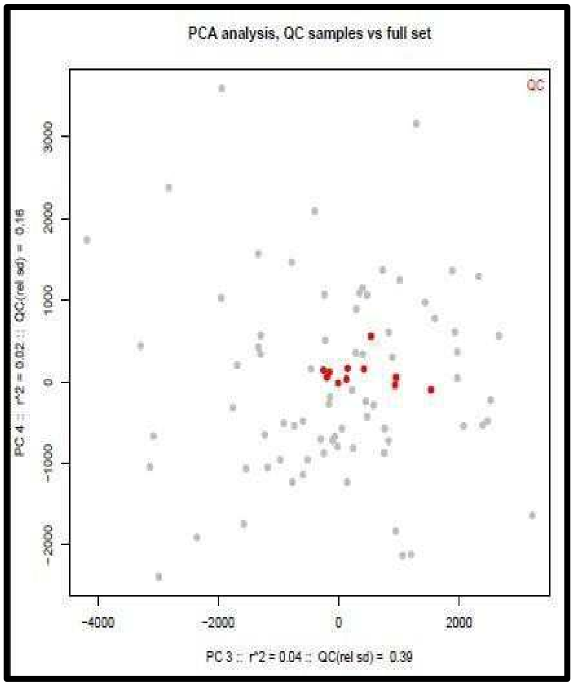
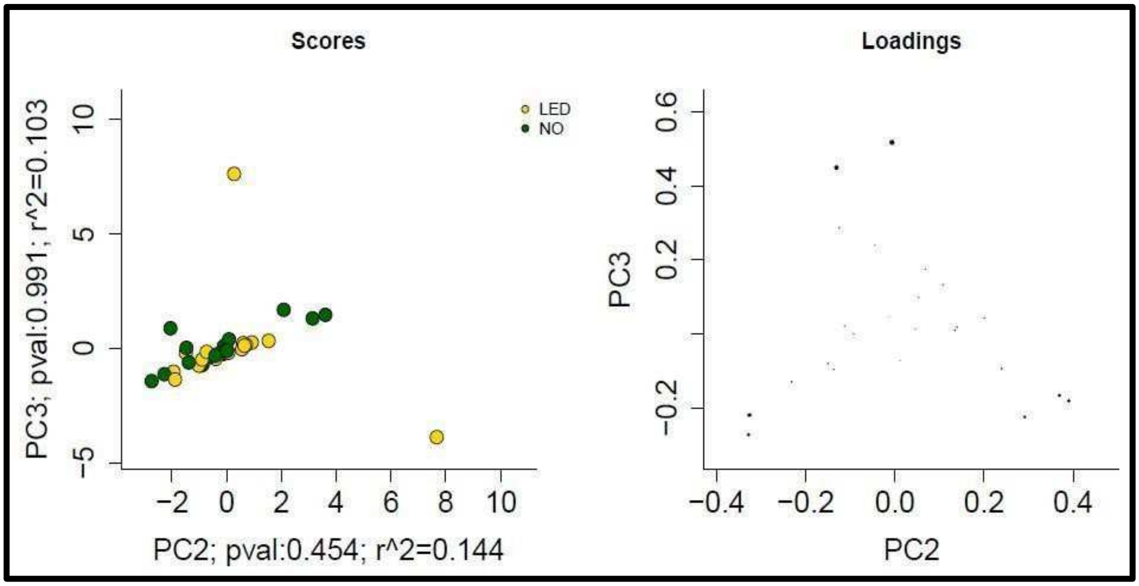


Figure 12: PCA analysis, QC samples vs. full set

In the statistical summaries, every element from the dataset was plotted for the Green and Gourmet Lettuce, Organic Fertilizer, Supplemental light, or Experiment final calculations. The calculation of statistical results is presented on the left panel for each element, andthe box-whisker plots allowed picture visualization of the trends of the data and calculations. The plots above or below are examples and they are included and available in the supplemental files and collection of raw data.



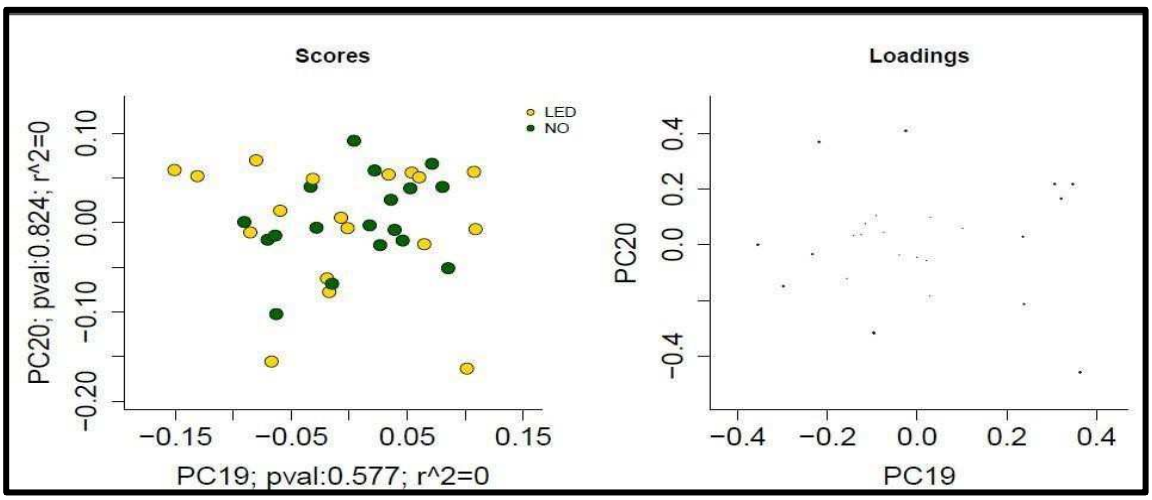


Figure 13: PC2 and 19PVAL

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
Lettuce	0.608612	0.136932	0.372207	0.573814	0.67924	0.094277	0.85529	0.204383	0.8132	0.000166	0.983952	0.303137
LED	0.049283	0.453761	0.991179	0.330194	0.873791	0.000891	0.951568	0.240798	0.991204	0.278725	0.181745	0.441605
Experiment	0.158696	0.393718	0.271709	0.41809	0.063794	0.157667	0.720233	0.719329	0.229256	0.594357	0.329173	0.111238

	PC13	PC14	PC15	PC16	PC17	PC18	PC19	PC20
Lettuce	0.237158	0.501399	0.276506	0.639632	0.236558	0.127539	0.503186	0.600063
LED	0.898687	0.423111	0.553253	0.347914	0.665588	0.01963	0.576763	0.823856
Experiment	0.72826	0.061416	0.607796	0.495248	0.501341	0.309767	0.670998	0.557555

Figure 14: PC1-PC20 outcomes

Detailed statistical ANOVAS and analysis

The previous raw data needed more detailed analysis. The following paragraphs, plots, charts, figures, pictures, and tables present that information. The researcher assisted with and actively ran the statistical analysis and all raw data used in available for re-analysis. A summary and discussion follows the charts, figures, etc.

Table 6: The significant type 1 SS (statistical significance) effects from running 25 3-way ANOVA with interaction effects

Element(dependent variable)	Source	DF	Type I SS	F Value	Pr > F
Be	Fertilizer	1	1997303	11.6	0.0011
S_34	Fertilizer	1	5.90E+14	7.58	0.0075
Mg	Fertilizer	1	1.60E+15	10.64	0.0017
Ca	Fertilizer	1	1.85E+14	9.92	0.0024
Ca	Lettuce_Type*Light	1	9.02E+13	4.83	0.0314
Al	Fertilizer	1	1.37E+15	13.12	0.0006
Al	Lettuce_Type*Light	1	6.55E+14	6.28	0.0146
V	Fertilizer	1	5.92E+08	8.36	0.0051
V	Lettuce_Type*Light	1	2.89E+08	4.09	0.0472
Cr	Fertilizer	1	7.33E+08	12.07	0.0009
Cr	Lettuce_Type*Light	1	6.38E+09	7.18	0.0092
Mn	Fertilizer	1	8.92E+10	12.16	0.0009
Fe	Fertilizer	1	2.47E+13	10.55	0.0018
Fe	Lettuce_Type*Light	1	1.06E+13	4.53	0.037
Cu	Fertilizer	1	3.09E+08	15.52	0.0002
Zn	Fertilizer	1	2.41E+10	5.68	0.02
Cr	Fertilizer	1	1.15E+10	30.98	< 0.0001
Mo	Fertilizer	1	35025860	60.82	< 0.0001
Pb	Fertilizer	1	3460445	7.81	0.0067
As	Fertilizer	1	3098.055	4.43	0.0391

Table (6) is a summary table for the significant Type I SS effects from running 25 3-ways ANOVA, one for each element. These 25 3-way ANOVA met the Assumptions of Homogeneity (AOH) of variance and Normality of Errors (NOE, Appendix (B)) shows the diagnostic plots and Type I SS for each ANOVA. Table (6) shows that Lettuce type*, Light two-way interaction effect is significant for the elements Ca, Al, V, Cr, and Fe, with p-values of 0.0314, 0.0146, 0.0472, 0.0092, 0.037, respectively. It also shows that Fertilizer main effect is significant for the elements Be, S_34, Mg, Ca, Al, V, Cr, Mn, Fe, Cu, Zn, Cr, Mo, Pb, and As, with p-values of 0.0011, 0.0075, 0.0017, 0.0024, 0.0006, 0.0051, 0.0009, 0.0009, 0.0018, 0.0002, 0.02, < 0.0001, < 0.0001, 0.0067, and 0.0391, respectively. Thus Fertilizer, which the researcher was not measuring or tracking as the primary IV or DV, emerged as a significant element in this research and more statistical outcomes were valid, reliable and Statistically Significant than the lettuce. Thus, while the research was important, the Null Ho should be rejected based on this table and we would fail to reject the Null Hypothesis if it were focused on fertilizer.

Running 25 ANOVA increased the type I error. One solution to control type I error is to reduce the dimension of the 25 elements (grouping) by using the Principle Component Analysis (PCA), and/ or the Factor Analysis (FA) to the centered raw data. Thus, we ran the ANOVA for each of the 25 elements.

Table 7: Eigenvalues, and the proportion of variation explained by the principal components

Component	Eigenvalue	Difference	Proportion	Cumulative
1	4.20E+14	2.85E+14	0.7038	0.7038
2	1.35E+14	1.10E+14	0.2263	0.9301
3	2.52E+13	1.18E+13	0.0422	0.9723
4	1.33E+13	1.09E+13	0.0224	0.9947
5	2.46E+12	2.03E+12	0.0041	0.9988
6	4.35E+11	2.82E+11	0.0007	0.9995
7	1.54E+11	4.27E+10	0.0003	0.9998
8	1.11E+11	1.08E+11	0.0002	1
9	3383297805	551811154	0	1
10	2831486651	2771749157	0	1
11	59737494.1	35212638.3	0	1
12	24524855.9	13514850.6	0	1
13	11010005.3	9319633.48	0	1
14	1690371.82	1179970.59	0	1
15	510401.229	146173.676	0	1
16	364227.553	119155.568	0	1
17	245071.985	97778.5489	0	1
18	147293.437	39106.2511	0	1
19	108187.185	36589.9758	0	1
20	71597.2097	27998.195	0	1
21	43599.0147	22825.8358	0	1
22	20773.1789	6498.58381	0	1
23	14274.5951	12657.068	0	1
24	1617.52708	1617.52708	0	1
25	0		0	1
Total	5.97E+14			

From Table 7 we find that the first two principal components explain 93.01% of the total variation. This is an acceptable percentage, and we would be satisfied by the first two principal components (2 groups).

Table 8: Pearson correlations, coefficients N=76

	Principal Component	
Element	**1**	**2**
Li_ctr	0.81855	-
Be_ctr	0.85811	-
Cd_ctr	0.26115	-
Se_ctr	0.76259	-
Na_ctr	0.94221	-
P_ctr	0.90225	0.35807
S_34_ctr	0.65061	0.64711
Mg_ctr	0.91285	-
K_ctr	0.85053	0.47948
Ca_ctr	0.89347	-
Al_ctr	0.81415	-
V_ctr	0.78633	-
Cr_ctr	0.77484	-
Mn_ctr	0.65757	-
Fe_ctr	0.81708	-
Co_ctr	0.37162	-
Ni_ctr	0.71469	-
Cu_ctr	0.89342	-
Zn_ctr	0.38336	-
Sr_ctr	0.81831	-
Mo_ctr	0.46871	-
Ba_ctr	0.49489	-
W_ctr	-0.00689	-
Pb_ctr	0.67532	-
As_ctr	0.85287	-

Table 8 shows the Pearson Correlations between the first two Principal Components and the Centered Elements. We consider an element with an absolute correlation magnitude of 0.5 and above is statistically significant to that principal component. From Table (8), we find that all elements are highly to moderately positively correlated to the first Principal component except for Cd, Co, Zn, Mo, Ba, and W_Ctr, Al, V, Cr, and Fe elements are moderately negatively correlated with the second principal component, and S_34 element is moderately positively correlated with the second principal component. S-34, Al, V, Cr, and Fe elements are correlated with both first and second principal components. Because of this overlapping of the importance of these elements to both the first and the second principal components, the interpretation of the first two principal components is very hard to explain. One solution to eliminate or reduce this overlapping of the importance of these elements to both the first and the second principal components is to use the FA-Principal Component Method with Varimax Rotation. The result of Type I SS from running two 3-ways ANOVA, one for each principle component scores (Appendix (C), is shown in Table (9), and Table (10).

Table 9: Type I SS for dependent variable: prin1

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	5.21E+13	5.21E+1	0.12	0.7253
Fertilizer	1	5.40E+14	5.40E+1	1.29	0.2595
Lettuce_T*Fertilizer	1	1.17E+12	1.17E+1	0	0.9579
Light	1	1.03E+15	1.03E+1	2.47	0.1208
Lettuce_Type*Light	1	7.53E+14	7.53E+1	1.8	0.184
Fertilizer*Light	1	3.47E+14	3.47E+1	0.83	0.3652
Lettuc*Fertili*Light	1	3.39E+14	3.39E+1	0.81	0.3707

Table 10: Type I SS for dependent variable: prin2

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	3.07794E+11	3.07794E+11	0	0.9525
Fertilizer	1	3.67E+15	3.67E+1	42.56	<.0001
Lettuce_T*Fertilizer	1	1.02E+14	1.02E+1	1.19	0.2799
Light	1	4.86E+13	4.86E+1	0.56	0.4554
Lettuce_Type*Light	1	1.78E+14	1.78E+1	2.07	0.1553
Fertilizer*Light	1	1.58E+14	1.58E+1	1.83	0.1808
Lettuc*Fertili*Light	1	1.09E+14	1.09E+1	1.27	0.2638

Table (9) & (10), show the Type I SS from using principle component scores of Prin1 & 2, (Appendix-B), as dependent variables. From Table (9) & (10), we find that there is a significant Fertilizer effect for the second principal component with p-value <0.0001. The rotated factor loading from using FA-Principal Component Method with Varimax Rotation to the centered elements is shown on Table (10).

Table 11: Rotated factor loading from factor analysis

	Factor	
Element	1	2
Li_ctr	0.85081	0.3036
Be_ctr	0.92424	0.32016
Cd_ctr	0.5885	-0.21446
Se_ctr	0.81693	0.27743
Na_ctr	0.68825	0.64585
P_ctr	0.38194	0.89523
S_34_ctr	0.01049	0.90876
Mg_ctr	0.82121	0.46433
K_ctr	0.28929	0.88305
Ca_ctr	0.83117	0.46397
Al_ctr	0.90124	0.25171
V_ctr	0.9531	0.18097
Cr_ctr	0.94397	0.17583
Mn_ctr	0.83871	0.17182
Fe_ctr	0.94279	0.2328
Co_ctr	0.61906	-0.03275
Ni_ctr	0.84342	0.18818
Cu_ctr	0.8805	0.4091
Zn_ctr	0.49092	0.01881
Sr_ctr	0.82274	0.36169
Mo_ctr	0.59691	0.10224
Ba_ctr	0.75173	0.00813
W_ctr	0.23236	-0.26937
Pb_ctr	0.89737	0.08438
As_ctr	0.85883	0.38963

From Table 11 Rotated Factor Loading Factor Analysis, the important elements to the first factor are: Li, Be, Cd, Se, Na, Mg, Ca, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Sr, Mo, Ba, Pb, and As. The important elements to the second factor are: Na, P, S_34, and K. We notice that we have only one element; which is Na that is important to both factors.

Table 12: Communality from factor analysis

Element	**Communality**
Li_ctr	0.82
Be_ctr	0.96
Cd_ctr	0.39
Se_ctr	0.74
Na_ctr	0.89
P_ctr	0.95
S_34_ctr	0.83
Mg_ctr	0.89
K_ctr	0.86
Ca_ctr	0.91
Al_ctr	0.88
V_ctr	0.94
Cr_ctr	0.92
Mn_ctr	0.73
Fe_ctr	0.94
Co_ctr	0.38
Ni_ctr	0.75
Cu_ctr	0.94
Zn_ctr	0.24
Sr_ctr	0.81
Mo_ctr	0.37
Ba_ctr	0.57
W_ctr	0.13
Pb_ctr	0.81
As_ctr	0.89
Total	18.53

Table 12 shows the communality values for each element from FA. The communality for a given element can be interpreted as a proportion of variation in that element explained by the first two factors. For example, 96% of the total variation in Be_ctr is explained by the first two factors.

Table 13: Type I SS for dependent variable: factor 1

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	0.227176	0.227176	0.27	0.6021
Fertilizer	1	13.89273	13.89273	16.78	0.0001
Lettuce_T*Fertilizer	1	0.283644	0.283644	0.34	0.5603
Light	1	0.194583	0.194583	0.24	0.6294
Lettuce_Type*Light	1	3.000841	3.000841	3.62	0.0612
Fertilizer*Light	1	1.103364	1.103364	1.33	0.2524
Lettuc*Fertili*Light	1	8.79E-05	8.79E-	0	0.9918

Table 14: Type I SS for dependent variable: factor 2

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	0.067413	0.067413	0.07	0.7919
Fertilizer	1	3.771503	3.771503	3.93	0.0516
Lettuce_T*Fertilizer	1	0.498758	0.498758	0.52	0.4737
Light	1	2.871017	2.871017	2.99	0.0884
Lettuce_Type*Light	1	0.129818	0.129818	0.14	0.7143
Fertilizer*Light	1	0.000319	0.000319	0	0.9855
Lettuc*Fertili*Light	1	2.339089	2.339089	2.43	0.1233

Tables 13 & 14, the Type I SS using factor scores as dependent variable from the first two factors, Appendix (D), shows that there is a highly significant Fertilizer effect for the first factor with p-value <0.0001, and a boarder significant Fertilizer effect for the second factor with p-value of 0.0516. Appendix (D), also, shows the fertilizer effect but not the Supplemental light vs. No supplemental light effect as statistically significant.

CHAPTER 5: CONCLUSION

The analysis of the effect of Fertilizer, Lettuce Type, and Supplemental light vs. No Supplemental light on the 25 measured elements involved considering three models. The first model is 3-way ANOVA for each element as a dependent variable. The result from this model showed that Lettuce type* Light two-way interaction effect is significant for the elements Ca, Al, V, Cr, and Fe, with p-values of 0.0314, 0.0146, 0.0472, 0.0092, 0.037, respectively. It also showed that Fertilizer main effect is significant for the elements Be, S_34, Mg, Ca, Al, V, Cr, Mn, Fe, Cu, Zn, Cr, Mo, Pb, and As, with p-values of 0.0011, 0.0075, 0.0017, 0.0024, 0.0006, 0.0051, 0.0009, 0.0009, 0.0018, 0.0002, 0.02, < 0.0001, < 0.0001, 0.0067, and 0.0391, respectively.

The drawback of this model as a result of running 25 ANOVA is a highly uncontrolled type I error. To control type one error, we considered grouping these 25 elements into two groups by using Principal Component Analysis (PCA). The result from PCA showed that there is a significant Fertilizer effect for the second principal component's scores with p-value<0.0001. However, this research focused on Supplemental lights vs. No Supplemental lights and fertilizer was changed to a DV in this study. Thus, the researcher concluded that while there is a significance for the five elements of Ca, Al, V, Cr, and Fe, with p-values of 0.0314, 0.0146, 0.0472, 0.0092, 0.037, respectively for the Supplemental lights vs. No Supplemental lights, it is not significant for all elements.

Likewise, even though the fertilizer main effect is significant for the 15 elements respectively, fertilizer was changed to a DV in this study, Supplemental lights vs. No Supplemental lights and the two lettuces (Green and Gourmet) were the IV sand DV's. Because of the overlapping of the importance of some of the elements to both the two first principal components, we considered the third model by using FA-Principal Component Method with Varimax Rotation to eliminate or reduce this overlapping. The result of regressing fertilizer, lettuce type, and light on first two factors' scores showed that there is a highly significant fertilizer effect for the first factor scores with p-value <0.0001, and a boarder significant fertilizer effect for the second factor scores with p-value of 0.0516.

Therefore, the researcher focused on the main effect and the overall importance of the body of research and the study: (1) there is a critical need to increase and improve the conditions to produce Green and Gourmet Lettuce in controlled agricultural and horticultural environments. The second focus (2) shows the need to improve the growth of lettuce in agricultural and horticultural environments in arid, hot, and dry, near waterless or limited water and desert areas globally. This improvement will allow large farmers and individual families to improve the quality of their lettuce production in various ways. The research will assist in improving lettuce growth where the growing seasons can be or are short and can be extended in the future via these improvements. Likewise, lettuce growth can be controlled and better regulated to increase the production of lettuce globally under severe conditions with less water, loss of the products and at lower costs to the producers, families, farmers, and others whether they are rich or poor. These improvements can also be generalized or used, with some modifications, on other vegetables.

Summary

The research proposal for this "Thesis: Evaluation of the Effectiveness of Supplemental lights vs. No Supplemental lights on Hydroponically Grown Lettuce" and subsequent research successfully examined the key literature research from the past 20 years regarding theories of how to plant, cultivate, grow, and harvest more lettuce for human consumption in controlled environments in arid and extremely dry areas, the research, and findings. There are a lot of historical factors and government research regarding growing lettuce but not using (1) Supplemental lights vs. No Supplemental lights and/or (2) the effects of fertilizers in growing lettuce with Supplemental lights vs. No Supplemental lights in greenhouse environments. It shows that it is possible to produce rich mineral and market ready lettuce in controlled agricultural and horticultural environments. This study did show that there is a critical need for the study and others like it using Supplemental lights vs. No Supplemental lights vs. Fertilizers and other DV's and IVs and the findings contributed to the overall body of literature in the fields of Agriculture and Horticulture in growing lettuce and other vegetables. However, more research needs to be done using various modifications.

The purpose of the study was to examine the literature from the past 20 years pertaining to evaluation of the effectiveness of Supplemental lights vs No Supplemental lights and two types of fertilizer: 1). Organic and 2). Synthetic fertilizers and its effect on two types of lettuce: 1) Green Salad Bowl Lettuce and 2) Gourmet Blend Mix. It was produced in an agricultural hydroponic greenhouse environment at the CSU. The researcher used the ICP-MS machine and common quantitative statistical analysis methods to evaluate the validity and reliability of the findings. The results should help agriculturalists, farmers, horticulturalists, policy makers, researchers, scientists, universities, and/or other key stakeholders to improve their lettuce growing, production, and marketing effectiveness globally.

The research and researcher showed that successfully growing of hydroponic organic lettuce (*Lactuca sativa)* in agricultural greenhouse settings under Supplemental lights vs No Supplemental lights or normal sunlight had little effect on the quality and quantity of the lettuce's mineral contents but the fertilizers had a statistically significant effect on the lettuce. During the research, the researcher and all involved showed that there are numerous benefits to growing the lettuce in controlled agricultural /horticultural environments under Supplemental lights, with fertilizers, limited water, where cost can be reduced, and where production time and conditions can be improved and/or extended in Colorado, the Middle East, and other dry and arid regions globally.

The purpose was fulfilled and the researcher tested the effects of the Supplemental lights vs. No supplemental lights and fertilizers on the two types of lettuce and their effects on the 25 traceable minerals. The Supplemental lights had no valid and reliable statistical effects on the quality, quantity, and effectiveness lettuce production with high mineral contents except on about 5 minerals but the fertilizer did have significant effects on about 20 minerals after two weeks of germination and six weeks of growth.

The mineral content with the ICP-MS machine was successful and valid and reliable statically data extracted from the final analysis and findings whether it supported the rejection or acceptance of the Null Hypothesis. The Null Hypothesis regarding the Supplemental lights was rejected but the effect of the organic and synthetic fertilizer was accepted, there was a valid and reliable effect. That does not mean the research and findings were meaningless because the researcher will present the significant main effect of the study which is of great value: (1) there is still and global need to improve the growth and production of lettuce under controlled Supplemental, non-Supplemental lights conditions in greenhouse conditions. (2) Such controlled environments are needed in all arid countries in the world (3) there is a shortage of more than 20 – 30 million tons of food for the overall populations involved. (4) The researcher will use the internal standard as part of the analysis. (5) The research adds to the body of agricultural and horticultural greenhouse research that is echo friendly and saves water, resources, time, extends growing seasons, improves methodologies for small and larger farmers and growers globally. (6) Finally, there is a critical need for additional research using additional and/or modifying the use of the IVs and DV's in the research.

The researcher tested various outcomes and data and mineral content of 76 samples of each variety of the lettuce under various conditions and fertilizer modifications. Thus, the Independent Variable (IV) of Supplemental lights vs. No Supplemental lights had less effects on the DV of lettuce. However, the fertilizer had a significant effect on the DV of lettuce and its rich mineral content. The conclusion is a critical need for greater lettuce production globally using Supplemental lights vs. No Supplemental lights or outside sunlight in arid areas and controlled greenhouse climates where water and light can be controlled. As researchers, farmers, agriculturalists, horticulturists, and others, we can change the historical method of greenhouse lettuce production and improve its production and marketability based on these findings and additional research.

The researcher did examine the research findings of studies and several theories. She compared hydroponic greenhouse lettuce production using Supplemental light s Vs No supplemental light using varied conditions. During the study, the advisors and professors recommended and altered the study to examine and track the effect of fertilizers (Organic vs. Synthetic) because of its likely effect on the outcomes, which proved to be true. As a result, the researcher did determine that the 25 different minerals in 76 heads of lettuce were successfully produced for human consumption but the Supplemental light effect was not statistically valid and reliable compared to the fertilizers.

The researcher did determine that hydroponically grown lettuce can be successfully produced and marketed in mass to meet the growing demand vs. traditional methods for growing lettuce in arid, extremely dry, and open sunlight outside with limited water sources. She also used the ICPMS Sample Digestion Materials to measure digestibility. Thus, the researcher concluded that while there is a significant effect for the five elements of Ca, Al, V, Cr, and Fe with Supplemental lights vs. No Supplemental lights with p-values of 0.0314, 0.0146, 0.0472, 0.0092, 0.037, respectively, it is not significant for all elements. Likewise, even though the fertilizer main effect is significant for the 15 elements respectively, fertilizer was changed to a DV in this study, Supplemental lights vs. No Supplemental lights and the two lettuces (Green and Gourmet) were the IV sand DV's. Fertilizers had a greater effect on the DV than the targeted Supplemental lights vs. No Supplemental lights.

Finally, the findings showed there is a critical need for additional research using various IVs and DV's such as Supplemental lights vs. No Supplemental lights, fertilizers, water levels, lettuce growth under Supplemental lights, sunlight, amounts of fertilizers, etc. These can be researched under various controlled agricultural / horticultural conditions to improve overall global yields during the extended growing seasons with fewer upsets to the growers, farmers, and plants. This research has contributed to the production of lettuce globally based on the findings in severe and highly arid or dry weather conditions and/or other factors that prevent its maximum growth and production.

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APPENDIX (A) RAW DATA

Obs	Analyte	Type	Fertilizer	Light	Li	Be	Cd	Se
1	L28-S13-	Gourmet	NON	LED	7190.00	1145.37	2547.74	5048.94
2	L67-S4-G	Gourmet	NON	LED	4510.43	742.23	666.49	2718.40
3	L09-O21-	Gourmet	Organic	LED	483.15	67.56	251.72	419.23
4	L55-O51-	Green	Organic	NO	2478.21	211.17	413.14	1426.42
5	L31-S50-	Green	NON	NO	6058.48	889.39	542.21	3255.39
6	L42-O3-G	Green	Organic	LED	2934.03	334.55	668.09	2201.06
7	L54-O39-	Green	Organic	NO	3263.79	425.65	2171.92	1934.96
8	L33-S42-	Green	NON	NO	5145.80	676.38	1167.89	3035.39
9	L29-S28-	Gourmet	NON	LED	1131.82	127.33	194.57	937.91

Obs	Na	P	S_34	Mg	K	Ca
1	5808665.37	8461003.06	13409658.81	35995622.48	42566154.79	17357813.83
2	3922561.39	9010076.11	17880961.23	28072557.08	41561802.43	11860617.88
3	146892.60	3199582.42	5092064.22	1568769.61	22683100.36	3068484.01
4	2253672.05	9344703.29	22856341.98	10897088.52	56860802.75	7653938.38

5	5413210.78	11203049.19	23675230.02	32384545.82	52965057.58	16975223.75
6	1969118.61	7040538.62	16807453.76	12912024.31	37825657.83	7653168.94
7	3199028.55	10322655.35	18898155.69	14962242.20	50005797.19	12457638.75
8	2886799.61	6703174.13	11150699.54	21457507.49	31221882.98	10887278.12
9	801859.29	6507603.83	12627334.65	11691073.40	37430727.13	6208287.79

Obs	Al	V	Cr	Mn	Fe	Co	Ni
1	40773939.49	30847.02	114293.59	316642.75	5329559.91	1998.91	9573.93
2	20974812.44	18898.71	53954.30	224308.58	2763487.23	1407.94	3833.76
3	270764.86	2406.69	9239.55	66677.11	506876.84	208.67	1203.78
4	3919664.94	6459.05	20926.91	103697.11	1036644.41	553.87	2788.06
5	33893787.44	23303.64	82762.90	355488.27	3703097.43	1162.76	4587.24
6	7735499.03	10715.83	33380.67	133552.75	1745948.18	775.66	3964.26
7	11472693.83	15368.65	47343.34	201348.12	2176214.95	1011.43	3601.82
8	18344688.23	18812.26	60390.01	210584.22	2792840.41	986.11	4264.70
9	2552546.22	4404.41	12438.29	107771.76	792165.06	278.04	1278.95

Obs	Cu	Zn	Sr	Mo	Ba	W	Pb	As
1	18412.46	173648.48	75429.62	2005.21	54352.28	839.137	2213.76	100.035
2	14144.57	168541.43	49732.23	1962.36	30726.85	262.223	1336.61	55.580
3	4177.26	40030.96	9033.09	392.89	6163.30	38.206	216.14	21.847
4	7961.39	81487.87	22785.44	641.97	15646.46	81.975	225.13	39.504
5	21287.50	188116.26	84384.05	2985.27	40035.53	462.154	1419.45	84.985
6	9326.12	120411.87	23469.01	582.17	21548.09	116.416	559.40	45.399
7	12042.63	116961.38	38793.06	942.72	24450.19	237.705	749.36	71.631
8	13210.18	201710.51	48065.71	2132.49	29420.01	280.304	1119.25	59.107
9	8321.35	119955.03	30085.36	1498.14	10335.10	59.696	340.45	27.722

Obs	Analyte	Lettuce_ Type	Fertilizer	Light	Li	Be	Cd	Se
10	L15-O38-	Green	Organic	NO	5347.73	607.52	452.14	3176.13
11	L39-S49-	Gourmet	NON	NO	6511.23	1002.92	2728.61	4746.07
12	L18-O36-	Gourmet	Organic	NO	7654.17	573.70	998.66	6047.13
13	L79-S44-	Gourmet	NON	NO	1518.54	150.14	247.27	1283.45
14	L03-O11-	Green	Organic	LED	2182.00	263.16	576.59	

1399.47

15 L52-O35- Green Organic NO 3489.15 485.60 518.79
3684.17

16 L69-S23- Gourmet NON LED 4963.52 793.63 951.39
3620.36

17 L19-O47- Gourmet Organic NO 2707.21 262.82 492.85
1750.40

18 L68-S8-G Gourmet NON LED 6548.76 1014.74 925.24
5071.49

Obs Na P S_34 Mg K

Ca

10 5927203.91 15455762.09 35823787.90 26295635.06 75937683.56
13187117.31

11 4947896.46 8343922.20 9637980.84 33396810.98 45313229.99
16274968.69

12 3385366.36 11774101.15 25711451.77 33292970.78 55215965.88
10901610.92

13 725219.91 6645775.99 10846990.88 12342858.04 31784696.97
5818548.17

14 2249458.39 8813981.27 20619536.68 9916683.25 42213202.33
8279555.14

15 3409995.02 9756135.19 18220208.08 17517323.14 49636886.54
8717659.78

16 3524111.25 9567279.16 15128728.79 31683932.07 44038200.89
12670705.77

17 3554819.36 9234551.62 24558238.83 15229210.57 47945891.19
8762235.47

18 4201288.33 9567113.56 16094694.19 33338669.47 40525328.58
15219719.44

Obs	Al	V	Cr	Mn	Fe	Co	Ni
10	17495687.76	15491.60	47231.18	214370.36	2295727.07	785.14	3970.67
11	33966488.87	30233.88	103749.31	243620.68	4653300.64	1624.42	7134.98
12	13933328.98	18615.25	52674.59	140535.19	3178123.30	1732.94	9421.97
13	3703920.72	5758.73	16681.18	112494.85	921729.08	361.44	1771.03
14	5717520.50	7555.22	22470.90	168646.70	1171410.61	375.56	1931.26
15	11547595.89	13023.27	36054.46	150036.52	1947505.81	764.19	4650.81
16	20266831.34	19985.67	59411.13	223141.70	2938652.04	1032.62	4956.93
17	7241837.80	8180.27	19458.13	117549.36	1158356.00	382.46	2126.20
18	28842315.02	24269.59	81562.17	301672.56	3925790.99	1361.22	6527.48

Obs	Cu	Zn	Sr	Mo	Ba	W	Pb	As
10	15643.45	135489.97	43863.96	880.62	22531.14	347.48	756.40	82.852
11	18069.32	163523.54	69566.81	2115.56	48245.08	369.32	2640.29	81.459
12	14154.27	123399.08	32189.30	727.92	45898.87	176.74	615.67	65.515
13	8007.55	100380.51	27209.51	1582.57	12815.48	69.63	260.22	25.374
14	9596.73	128536.20	24943.80	609.14	11797.47	122.33	398.63	47.619

15	10565.88	108520.20	30305.09	744.23	22929.23	139.64	765.76	54.994
16	15238.47	159365.76	56438.79	2222.73	34555.96	252.50	1284.06	64.143
17	8608.42	94164.28	28110.07	586.66	11546.36	97.15	567.00	42.941
18	17397.95	163934.22	67139.21	2826.60	46970.85	377.12	1407.91	76.409

Obs	Analyte	Lettuce_ Type	Fertilizer	Light	Li	Be	Cd	Se
19	L36-S53-	Gourmet	NON	NO	1148.11	152.25	679.33	917.30
20	L06-O23-	Gourmet	Organic	LED	1506.19	130.63	460.98	1200.16
21	L12-O35-	Green	Organic	NO	11004.74	1347.75	1203.82	8808.25
22	L01-O6-G	Green	Organic	LED	3058.53	493.85	1383.58	2145.38
23	L05-O25-	Green	Organic	LED	3255.40	342.24	121.11	2760.35
24	L48-O12-	Gourmet	Organic	LED	3982.59	544.09	422.13	2597.91
25	L51-O44-	Green	Organic	NO	3501.04	352.47	506.21	2328.98
26	L41-O15-	Green	Organic	LED	5317.28	674.71	983.09	4484.64
27	L23-S26-	Green	NON	LED	1095.19	67.56	116.23	1084.16

Obs Na P S_34 Mg K Ca

19	743917.99	4983682.52	7373955.88	8966858.04	28179939.89	5324189.25
20	1337924.99	5227990.14	10700490.07	6426358.03	28706806.15	5277439.87
21	5954617.99	9879691.79	14825837.68	42581612.94	44011990.76	15789544.80
22	2622947.29	6684979.21	18256865.22	13269982.69	36750169.71	8448313.09
23	2538032.01	10782900.21	37141560.50	17186014.38	45012387.28	7796932.20
24	4155733.72	9943172.62	36559267.04	20401674.46	44413353.50	9333193.78
25	2888441.39	8890149.21	22290340.13	15210531.27	36948528.61	8146012.00
26	3291632.64	8918583.07	23616088.72	22479771.35	38174089.50	10570652.77
27	549995.06	7158293.59	19199314.98	16139197.37	31743057.85	6751381.88

Obs	Al	V	Cr	Mn	Fe	Co	Ni
19	4097281.58	6064.77	15849.18	118856.36	870436.68	279.09	1327.06
20	3525489.06	5694.47	15789.77	104618.32	921791.88	595.99	1523.79
21	40759513.71	33180.87	119135.72	316002.90	5981773.51	3105.06	11641.76
22	11243441.44	14243.22	35710.68	155271.73	1923632.84	579.81	2717.08
23	3486111.40	8795.27	15654.99	137099.67	1070415.15	509.15	3061.64

24	12782285.28	12672.53	31415.77	156372.95	1784589.20	831.12	2788.36
25	8718626.91	9774.33	28612.07	150112.76	1480285.29	560.69	2894.45
26	15129477.75	16868.24	52314.24	191610.06	2582242.24	1144.93	6058.74
27	855360.55	2388.07	10777.84	124306.61	667702.52	305.05	1637.30

Obs	Cu	Zn	Sr	Mo	Ba	W	Pb	As
19	7627.22	93381.05	26112.44	1538.17	9879.16	88.61	475.95	29.805
20	6860.99	81800.78	17052.58	529.48	10563.59	85.52	386.15	29.085
21	18908.19	194117.13	56294.71	1308.67	65948.31	615.18	2164.75	101.669
22	9957.24	98899.09	29748.15	611.82	19557.04	172.89	910.85	51.796
23	10939.96	152509.15	22522.55	786.32	15313.54	523.24	183.32	42.633
24	11163.65	131094.68	31242.53	686.01	18863.05	156.03	925.31	57.710
25	9996.52	96254.35	25432.60	696.65	17301.16	140.45	496.04	47.587
26	13187.21	116474.24	34958.14	621.52	33777.97	200.74	705.03	63.642
27	8579.38	130969.87	34808.36	1857.27	8624.54	43.11	183.59	21.697

Obs	Analyte	Lettuce_ Type	Fertilizer	Light	Li	Be	Cd	Se

28	L26-S1-G	Gourmet	NON	LED	9620.58	1545.57	1526.72	5968.40
29	L04-O27-	Green	Organic	LED	4054.77	266.36	165.34	3421.33
30	L58-O34-	Gourmet	Organic	NO	4894.58	687.36	1842.28	4448.65
31	L22-S11-	Green	NON	LED	9541.17	1491.34	1665.09	7631.96
32	L45-O27-	Green	Organic	LED	3911.26	373.76	510.31	2375.24
33	L20-O44-	Gourmet	Organic	NO	8598.27	761.55	420.25	8175.58
34	L65-S27-	Green	NON	LED	6433.24	948.75	1420.70	4977.86
35	L43-O9-G	Green	Organic	LED	1856.38	180.15	826.60	1354.78
36	L35-S35-	Green	NON	NO	836.47	67.56	168.51	879.27

Obs	Na	P	S_34	Mg	K	Ca
28	7282207.02	12073795.19	16521538.64	42574109.43	58781103.07	22587457.71
29	1390321.46	6778672.42	18343385.41	15415727.98	33422923.43	5228820.48
30	5002739.59	13935395.48	26619285.84	27433292.41	63150899.61	12706525.79
31	7476300.61	10902668.81	19800131.93	40633528.02	51436990.20	16302008.96
32	3889427.06	11479940.22	42219584.25	17989077.74	54462274.82	8137410.01
33	3740974.62	10084832.83	26757818.88	39364834.73	47737592.86	8768605.68

34	2978718.43	9228673.43	14303925.03	29703397.11	44353661.45	11388675.44
35	1768755.17	7014918.93	21058462.42	9198307.48	35787943.81	6836317.64
36	390694.73	5370908.28	13032893.45	7679429.04	27518907.27	4742149.95

Obs	Al	V	Cr	Mn	Fe	Co	Ni
28	45594790.27	37195.75	137410.22	411731.68	6536734.01	1843.33	7245.81
29	4347111.50	8326.43	20365.03	100395.53	1322743.68	791.92	4316.81
30	16785189.30	18534.95	41520.68	201942.06	2708611.31	870.85	4667.15
31	42025666.77	34880.12	102967.50	303153.99	5951599.43	1897.74	8794.82
32	8102821.45	9043.89	23184.96	141109.68	1385386.52	569.18	2698.60
33	15262212.15	14960.54	57548.52	143809.93	2818832.54	1583.19	8948.05
34	18879307.00	23195.34	65009.02	258927.53	4159568.03	1751.48	6544.51
35	4085157.15	6945.93	19211.14	120246.45	999554.61	366.06	1926.39
36	1153238.12	2583.17	9983.14	102978.37	515236.03	192.95	1268.41

Obs	Cu	Zn	Sr	Mo	Ba	W	Pb	As
28	23429.52	181585.07	95188.54	2637.93	63946.66	607.39	2451.78	115.658

29	8238.02	89818.64	16266.66	510.63	20927.73	68.20	158.08	42.460
30	14928.49	156733.88	49659.98	1343.05	30677.93	217.70	1244.67	76.497
31	18802.28	160103.71	65457.93	903.81	58734.29	510.73	2852.69	102.914
32	10775.99	135426.10	28742.44	615.38	14707.12	195.61	376.98	46.503
33	15049.45	157464.82	32430.37	721.04	37902.28	329.50	789.27	106.577
34	17145.42	147647.78	54259.90	2004.99	44815.09	614.60	1114.45	110.957
35	7941.35	89475.33	23111.13	533.32	10069.91	106.58	329.25	42.765
36	7107.03	94861.37	24252.77	1338.02	6626.29	46.56	178.05	21.722

Obs	Analyte	Lettuce_ Type	Fertilizer	Light	Li	Be	Cd	Se
37	L32-S46-	Green	NON	NO	2904.30	492.62	477.59	2702.43
38	L74-S43-	Green	NON	NO	3677.54	581.29	989.80	3271.10
39	L63-S11-	Green	NON	LED	5715.97	961.73	904.24	4231.30
40	L13-O54-	Green	Organic	NO	6119.75	828.93	776.65	4186.72
41	L77-S53-	Gourmet	NON	NO	3020.57	500.93	717.94	3291.27
42	L76-S60-	Gourmet	NON	NO	7835.63	1313.07	519.37	8988.31
43	L37-S55-	Gourmet	NON	NO	3805.43	616.49	1512.86	3009.76

44	L49-O30-	Gourmet	Organic	LED	1631.37	177.51	610.46	1315.21
45	L71-S32-	Green	NON	NO	7022.07	1007.21	2418.39	6195.59

Obs	Na	P	S_34	Mg	K	Ca
37	1921771.33	7339052.96	19312955.58	15494365.72	32784341.38	7697771.89
38	2938344.22	8109801.88	17624664.04	21257545.77	32017197.74	11013831.75
39	4811602.58	11801454.91	30287427.12	37002248.25	44275493.95	14277445.18
40	5413979.98	14008744.20	27389398.19	25758364.74	62227573.18	14163256.10
41	2369550.67	7145244.98	13434609.71	19530824.28	33956101.60	7566588.29
42	4468861.46	13004955.87	32163862.82	51297706.04	50232076.03	13731850.39
43	3535568.34	11888740.64	23804232.15	28755591.22	53662517.83	12449488.69
44	1872678.80	6422315.57	20801356.00	9088589.79	37148604.16	6218878.04
45	5981674.13	13950328.88	32443231.64	33328093.02	55861409.58	15857925.22

Obs	Al	V	Cr	Mn	Fe	Co	Ni
37	8934011.60	10887.81	33861.45	160978.22	1592948.79	683.54	3096.50
38	15980631.09	15045.48	45631.80	237214.05	2207771.52	593.12	2781.97
39	22736785.63	18716.56	56588.83	290890.61	2993720.03	2295.44	4086.29
40	19613252.95	20788.80	56735.31	259534.36	3157476.30	995.34	

4816.01

41	11352961.51	12592.87	28775.20	156543.53	1705769.22	490.54	2529.15
42	26200587.31	24429.04	70855.24	271778.50	4393764.12	1946.61	8848.90
43	15024201.41	15372.79	36616.04	230446.15	2344179.39	589.70	2912.10
44	4419784.21	6762.56	16511.22	112502.32	989795.38	270.86	1505.95
45	23536235.26	21309.58	69339.44	282155.56	3576810.37	1464.40	6734.35

Obs	Cu	Zn	Sr	Mo	Ba	W	Pb	As
37	9849.17	119605.65	32428.83	1255.44	16471.37	156.89	485.22	46.333
38	13813.99	153038.39	53702.70	2520.76	23820.43	262.61	812.41	53.732
39	17569.87	186782.87	70579.39	2991.51	28416.64	311.91	1062.36	71.152
40	16757.86	144511.32	51466.11	1060.27	30378.49	305.04	1140.16	87.339
41	11133.12	136321.84	40206.42	1475.02	18654.36	143.04	884.70	45.707
42	20513.72	209273.43	70503.86	2810.67	48508.49	320.69	1268.01	85.761
43	15775.88	204253.27	68513.14	2930.31	22014.10	221.82	1309.78	67.648
44	7680.74	87201.78	22584.81	648.83	8895.13	91.84	517.32	39.820
45	17051.17	148878.76	59403.17	1382.54	40847.53	357.12	1171.73	113.156

Obs	Analyte	Lettuce_ Type	Fertilizer	Light	Li	Be	Cd	Se
46	L34-S33-	Green	NON	NO	8832.83	1460.77	780.80	

9333.38
47   L47-O18- Gourmet   Organic   LED   3892.34   451.62 1201.68
3367.80
48   L62-S6-G Green   NON   LED   8250.62 1289.14 643.42
6534.47
49   L27-S22- Gourmet   NON   LED   6248.28   896.57 1683.89
6579.75
50   L72-S52- Green   NON   NO   8825.09 1219.81 1479.48
6245.40
51   L53-O55- Green   Organic   NO   8594.98 1235.92 2298.19
8147.75
52   L80-S55- Gourmet   NON   NO   2517.59   243.55 890.29
2381.54
53   L17-O59- Gourmet   Organic   NO   4938.98   250.25 414.73
1956.78
54   L08-O16- Gourmet   Organic   LED   9340.88   358.41 704.00
4388.90
Obs   Na   P   S_34   Mg   K   Ca
46   4979278.58 10375506.43 24748221.49 46556580.03 41914233.63
14619318.45
47   4124224.33   9176315.63 26693503.45 19303449.00 45785148.12
9106919.84
48   5166781.95 11400893.18 23053761.80 43915129.28 48029489.85
17814382.32
49   2732446.76   4926291.43   8225401.31 27472037.68 22846627.28
8828825.35
50   5502116.89 13562636.89 23941482.58 32302342.32 60782508.81
15193080.21
51   4701468.84   7686831.05 11131381.32 33816170.36 41127036.35
14103516.49
52   1504173.49   5191306.94 10712029.35 12068606.17 30473366.71
5451749.75
53   3957634.81 10519214.41 28984848.69 18838173.40 55601521.76
8491248.17

54	1352182.70	5417698.33	13034323.56	15488081.70	33162636.57	4985662.34

Obs	Al	V	Cr	Mn	Fe	Co	Ni
46	29701130.18	25686.07	86900.97	296272.97	4780812.45	3798.27	9183.17
47	12700204.89	13910.65	32371.63	154545.80	1951230.84	556.40	2816.53
48	29397214.78	26336.46	94524.46	360372.18	4903417.76	1543.77	7632.69
49	20061389.84	21098.07	63358.87	229519.68	3449042.31	1288.48	6803.97
50	24870206.81	26340.73	79081.53	291093.38	4385494.21	1772.99	7518.86
51	27431170.88	35245.74	100876.43	263276.28	5889579.74	2434.72	11474.03
52	6422007.84	9337.50	26022.45	120633.48	1332420.41	484.45	2506.79
53	6264270.81	7564.92	16995.40	127730.39	1125017.54	392.65	2224.39
54	5903090.59	10167.61	48481.19	112890.69	1695066.65	908.60	5161.27

Obs	Cu	Zn	Sr	Mo	Ba	W	Pb	As
46	19886.51	174233.03	68339.53	2636.08	54323.32	546.60	1464.81	97.418
47	11085.72	112551.59	34291.51	602.69	19138.65	164.98	1006.98	55.729
48	21852.04	183145.45	86605.36	3243.31	51734.86	439.80	1575.33	96.122
49	14944.00	122613.66	41181.88	1482.52	41829.36	321.72	1049.42	73.592

50	18934.19	139753.34	56816.21	1121.90	48964.66	391.59	1428.29	107.572
51	18434.02	193510.33	53277.86	825.70	74373.45	341.78	2350.69	115.470
52	9175.79	91321.80	26255.45	995.95	26749.55	162.81	527.27	46.334
53	9968.35	209604.73	34107.15	986.98	11553.08	1095.05	606.23	46.714
54	9832.22	412530.21	16279.02	1404.58	25405.13	5258.68	468.32	32.644

Obs	Analyte	Lettuce_ Type	Fertilizer	Light	Li	Be	Cd	Se
55	L59-O56-	Gourmet	Organic	NO	3744.46	554.93	1294.66	3085.13
56	L25-S9-G	Green	NON	LED	1278.58	67.56	343.79	1486.03
57	L56-O37-	Gourmet	Organic	NO	2068.74	194.21	657.42	1569.82
58	L02-O7-G	Green	Organic	LED	3648.15	424.33	672.75	3045.03
59	L11-O53-	Green	Organic	NO	3659.07	464.92	2187.41	3201.23
60	L40-S38-	Gourmet	NON	NO	3789.48	555.23	605.39	4475.91
61	L16-O49-	Gourmet	Organic	NO	3100.50	242.21	165.06	1926.90
62	L24-S16-	Green	NON	LED	7977.38	308.56	1558.34	2011.59
63	L75-S48-	Green	NON	NO	7072.29	1101.56	1180.73	6092.60

Obs	Na	P	S_34	Mg	K	Ca
55	3701108.72	8597719.70	21304478.51	16726594.45	45160154.29	9903372.95
56	146892.60	4494828.95	10603643.19	10720448.55	21732471.14	4675397.21
57	2982411.36	7704339.81	23813103.42	12298663.94	41880049.12	7440846.79
58	3561959.65	10525957.77	23695937.41	18915000.44	55853298.44	7943003.98
59	1216230.59	1575601.92	229568.44	11435036.52	11038254.49	5058863.64
60	2590932.66	8745412.23	20354061.06	27462736.57	44080432.89	8888315.52
61	4245952.70	11854425.60	30958116.85	19730404.02	64625671.48	8129020.52
62	1209397.98	5377053.33	9121500.45	13276503.86	27166462.93	9544852.97
63	5341454.04	10124743.21	22635483.49	32124931.56	47776056.91	15862885.10

Obs	Al	V	Cr	Mn	Fe	Co	Ni
55	12292079.21	15610.24	35038.08	178909.22	2108801.60	622.85	3335.13
56	2560806.81	4490.56	14910.75	119464.94	924543.13	369.38	2017.67
57	4676667.25	6349.91	15061.81	115286.88	865324.41	283.16	1765.69
58	9111533.76	11808.88	31359.79	151157.05	1635928.57	615.08	3365.79
59	11308151.46	17290.19	51131.78	140348.86	2277230.62	854.31	3867.17

60	12269450.72	13069.32	33483.20	170788.84	1986083.97	789.96	3932.22
61	5792001.60	7529.21	17395.57	126930.47	1100651.69	430.28	2488.31
62	8601141.28	12492.37	38144.69	207054.51	1754038.29	614.55	2864.24
63	23480772.50	23570.15	84432.21	360480.72	4101699.93	1482.18	6526.07

Obs	Cu	Zn	Sr	Mo	Ba	W	Pb	As
55	11809.21	122951.77	36073.93	699.43	20127.92	214.99	1026.19	59.996
56	8266.93	97600.77	26268.05	1547.41	10736.37	125.52	213.80	24.076
57	7893.67	95738.82	24835.06	669.12	8815.06	81.13	445.21	42.444
58	11513.24	122532.65	31712.37	679.82	17764.97	175.33	579.55	59.852
59	8387.91	46895.22	18833.01	403.26	29199.25	175.85	732.00	40.883
60	12399.52	159766.89	48017.40	2102.03	24864.54	135.04	835.14	51.898
61	10914.55	116479.56	34063.93	777.48	11144.69	84.75	439.25	47.135
62	13000.82	446313.49	48091.16	2995.55	22585.95	4166.69	855.71	57.185
63	18813.89	182171.02	63266.42	1795.78	44947.20	441.75	1484.58	103.066

Obs	Analyte	Lettuce_ Type	Fertilizer	Light	Li	Be	Cd	Se

64	L44-O4-G	Green	Organic	LED	1383.32	450.82	235.14	6973.79
65	L07-O19-	Gourmet	Organic	LED	2844.48	310.55	876.93	2305.78
66	L64-S16-	Green	NON	LED	2549.12	386.53	1273.83	2196.91
67	L14-O40-	Green	Organic	NO	8047.79	779.58	418.04	9650.93
68	L70-S7-G	Gourmet	NON	LED	6900.34	1112.57	576.05	5878.30
69	L30-S20-	Gourmet	NON	LED	6809.08	860.08	1480.75	6238.15
70	L61-S22-	Green	NON	LED	1698.50	238.82	1293.44	1413.30
71	L66-S2-G	Green	NON	LED	4761.88	769.42	1514.68	5111.18
72	L38-S37-	Gourmet	NON	NO	7893.38	1026.31	701.34	9956.57

Obs	Na	P	S_34	Mg	K	Ca
64	1359244.64	6813544.33	21407833.15	6882921.83	38317826.98	6093224.55
65	3244931.42	8926491.49	24079141.41	14833446.31	48350813.79	9609512.86
66	1470044.54	4196655.24	6456992.98	12310863.34	19328220.20	6916445.72
67	3413354.47	13983563.70	27827085.47	34720505.58	68461179.06	10298873.28
68	5633102.15	12483441.79	24145660.21	39972169.98	57164188.12	14890849.02
69	4118951.92	9889257.89	23688571.54	28314361.74	44224192.71	12501859.91

70	760349.19	3597835.72	4680802.23	7927536.70	18161621.21	5261488.01
71	3118523.16	7447593.08	9663751.81	24794537.11	45077066.80	10689306.77
72	3052907.03	8211689.29	16712476.34	46303017.91	44201489.47	10692086.40

Obs	Al	V	Cr	Mn	Fe	Co	Ni
64	1730955.58	17968.52	12106.23	546171.86	828846.50	8586.89	8896.97
65	8080389.18	10506.01	26050.25	175892.77	1569096.70	500.99	2533.31
66	9431019.90	12815.05	37951.58	173841.00	1688609.96	547.48	2599.96
67	13977194.80	18952.59	52840.68	170976.52	3244182.85	1648.01	12203.86
68	26298655.34	25217.44	65521.31	275348.23	3844650.37	1288.45	5475.55
69	18975009.75	23186.50	68644.29	239503.41	3473296.84	1501.74	7835.25
70	5916510.73	10440.91	23763.80	116312.05	1291569.31	371.37	1673.75
71	18086992.27	21674.46	53535.24	192815.45	2992042.15	1024.29	5262.97
72	21807288.97	22416.07	67526.24	197624.90	4120375.75	2067.96	11105.88

Obs	Cu	Zn	Sr	Mo	Ba	W	Pb	As
64	8375.65	46947.67	17765.26	605.79	175395.85	166.06	2348.57	60.526
65	11516.72	139754.07	35337.83	815.20	15156.06	221.36	720.25	55.089

66	10039.34	84798.72	32334.43	1364.89	19081.55	236.51	625.20	42.635
67	15029.58	172335.84	37637.81	701.99	46730.62	151.04	474.94	84.690
68	20153.26	215393.22	79383.37	2963.07	36831.95	345.27	1893.55	85.446
69	17169.94	145979.45	48850.47	958.99	41346.38	343.82	1031.15	87.856
70	7927.15	86554.90	23999.09	975.40	13448.18	154.03	670.42	43.803
71	14729.95	315308.96	53340.67	1630.20	34622.51	204.38	1548.48	68.647
72	16168.88	165400.94	53178.67	1912.17	51404.05	326.30	1141.44	62.661

Obs	Analyte	Lettuce_ Type	Fertilizer	Light	Li	Be	Cd	Se
73	L78-S39-	Gourmet	NON	NO	8054.75	1400.23	1372.87	7427.13
74	L21-S30-	Green	NON	LED	8511.10	1281.32	1166.82	7991.88
75	L73-S59-	Green	NON	NO	11808.07	1984.04	820.82	10139.22
76	L46-O26-	Gourmet	Organic	LED	11623.86	1495.01	1591.05	10112.48

Obs	Na	P	S_34	Mg	K	Ca
73	5840689.09	11017119.46	16562667.68	38726066.86	55666942.13	17300250.82
74	5966910.05	11119744.67	29269019.56	43255726.65	52419260.02	17819550.56
75	7412517.91	18345615.98	36197519.63	63078987.84	71078080.59	

28327970.65

76 9256689.27 15756714.31 52431813.62 49083232.47 82345187.14 18130079.27

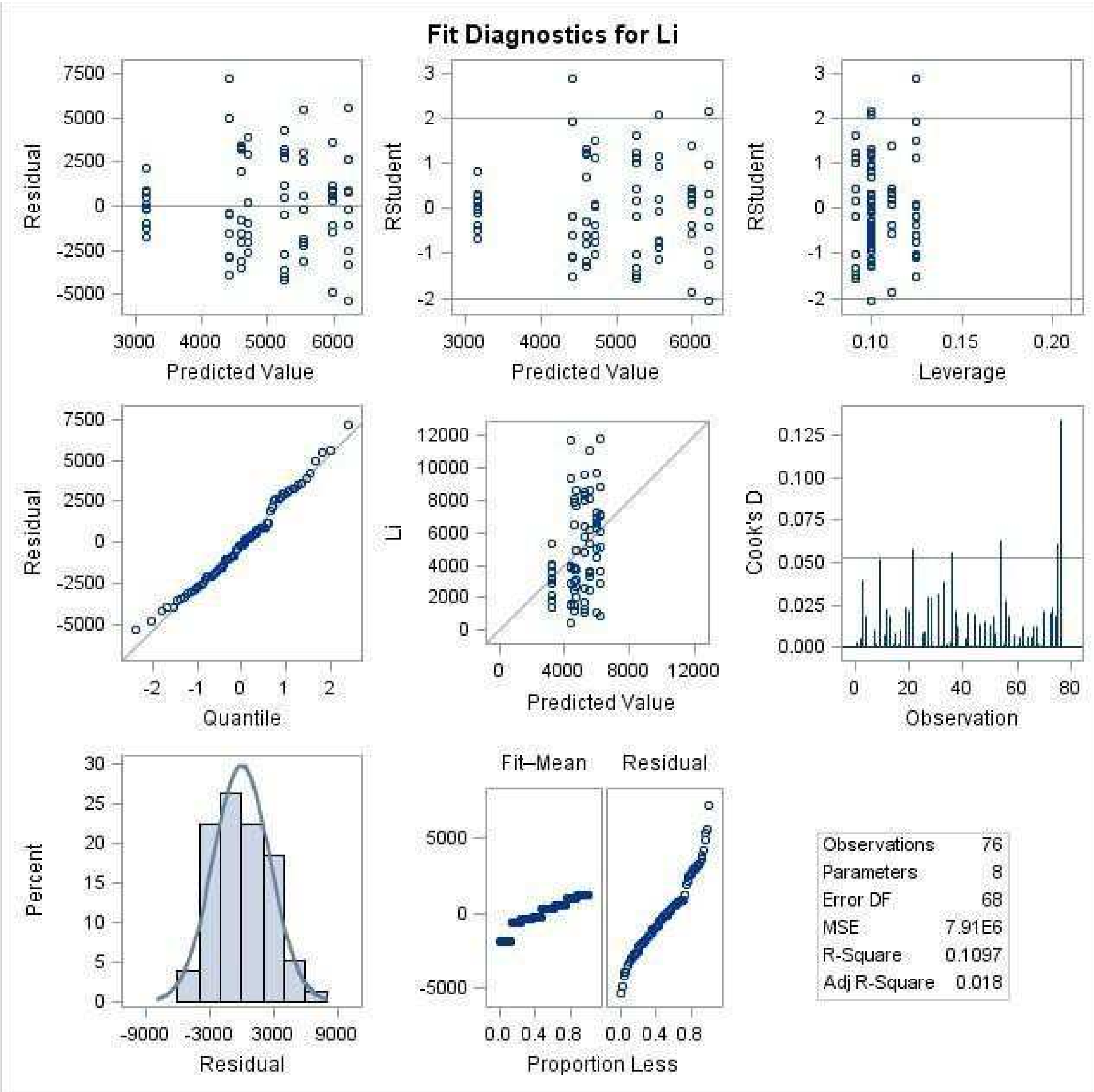
Obs	Al	V	Cr	Mn	Fe	Co	Ni
73	25416157.79	30411.48	95500.48	298923.43	5148859.63	1878.26	8403.52
74	28341341.08	26178.84	90946.18	327753.65	4661498.07	1596.82	7672.50
75	34735294.19	31963.78	156229.25	474159.68	7300912.28	2610.56	13871.20
76	34630215.31	33380.40	90095.06	265105.07	5168199.97	1791.62	7866.87

Obs	Cu	Zn	Sr	Mo	Ba	W	Pb	As
73	19407.65	175816.18	79100.92	2300.05	55690.40	409.60	2614.15	102.067
74	20323.52	192110.01	79711.47	2537.48	48083.96	449.61	1514.49	100.283
75	29458.94	230701.18	135043.04	6613.36	79116.98	664.53	2253.74	138.746
76	19714.39	194459.62	72330.50	879.84	70384.11	366.88	2259.06	102.610

APPENDIX (B) INDIVIDUAL 3-WAY ANOVA FOR EACH ELEMENT & DIAGNOSTIC PLOTS

Dependent Variable: Li

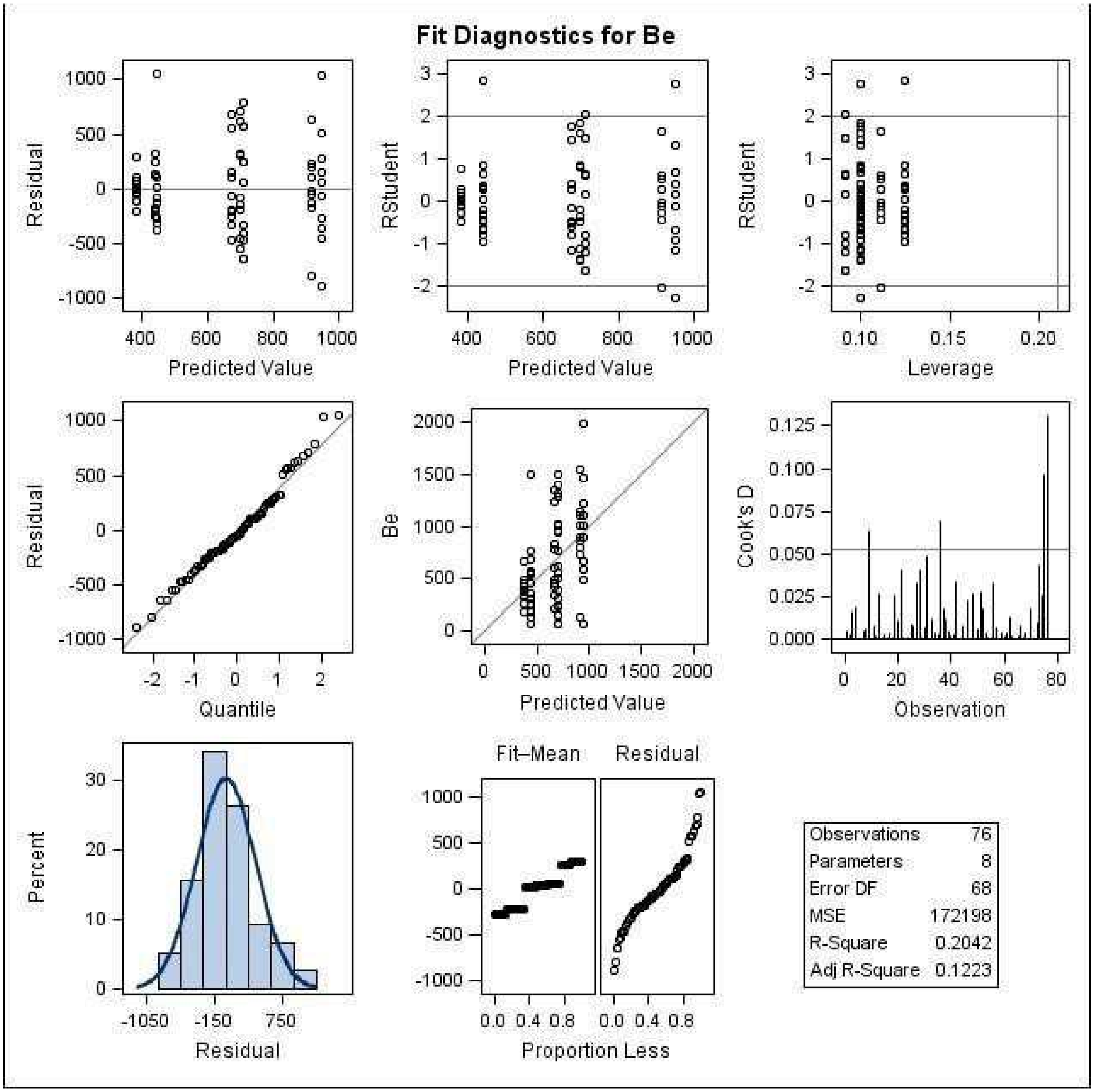
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	218604.59	218604.59	0.03	0.8684
Fertilizer	1	21143258.08	21143258.08	2.67	0.1066
Lettuce_T*Fertilizer	1	2034259.25	2034259.25	0.26	0.6136
Light	1	7152263.94	7152263.94	0.90	0.3449
Lettuce_Type*Light	1	24328058.64	24328058.64	3.08	0.0839
Fertilizer*Light	1	11277025.54	11277025.54	1.43	0.2365
Lettuc*Fertili*Light	1	75891.66	75891.66	0.01	0.9222



Dependent Variable: Be

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	34671.541	34671.541	0.20	0.6551
Fertilizer	1	1997302.635	1997302.635	11.60	0.0011
Lettuce_T*Fertilizer	1	18261.693	18261.693	0.11	

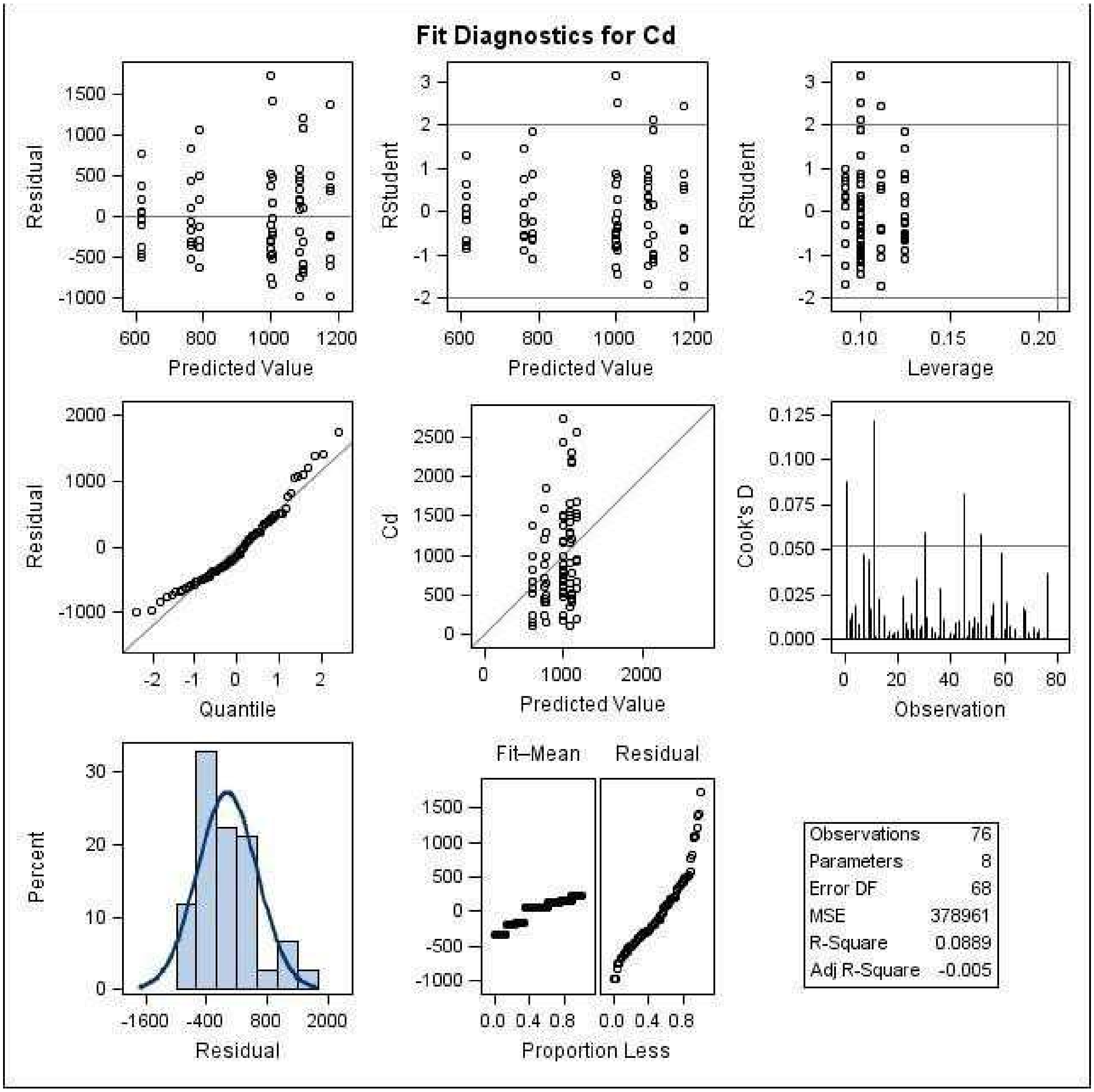
```
0.7457
Light                         1     147294.993     147294.993       0.86
0.3583
Lettuce_Type*Light            1     696818.066     696818.066       4.05
0.0482
Fertilizer*Light              1      80096.632      80096.632       0.47
0.4975
Lettuc*Fertili*Light          1      31040.478      31040.478       0.18
0.6725
```



Dependent Variable: Cd

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	2145.760	2145.760	0.01	0.9402
Fertilizer	1	1114599.361	1114599.361	2.94	0.0909
Lettuce_T*Fertilizer	1	62632.343	62632.343	0.17	

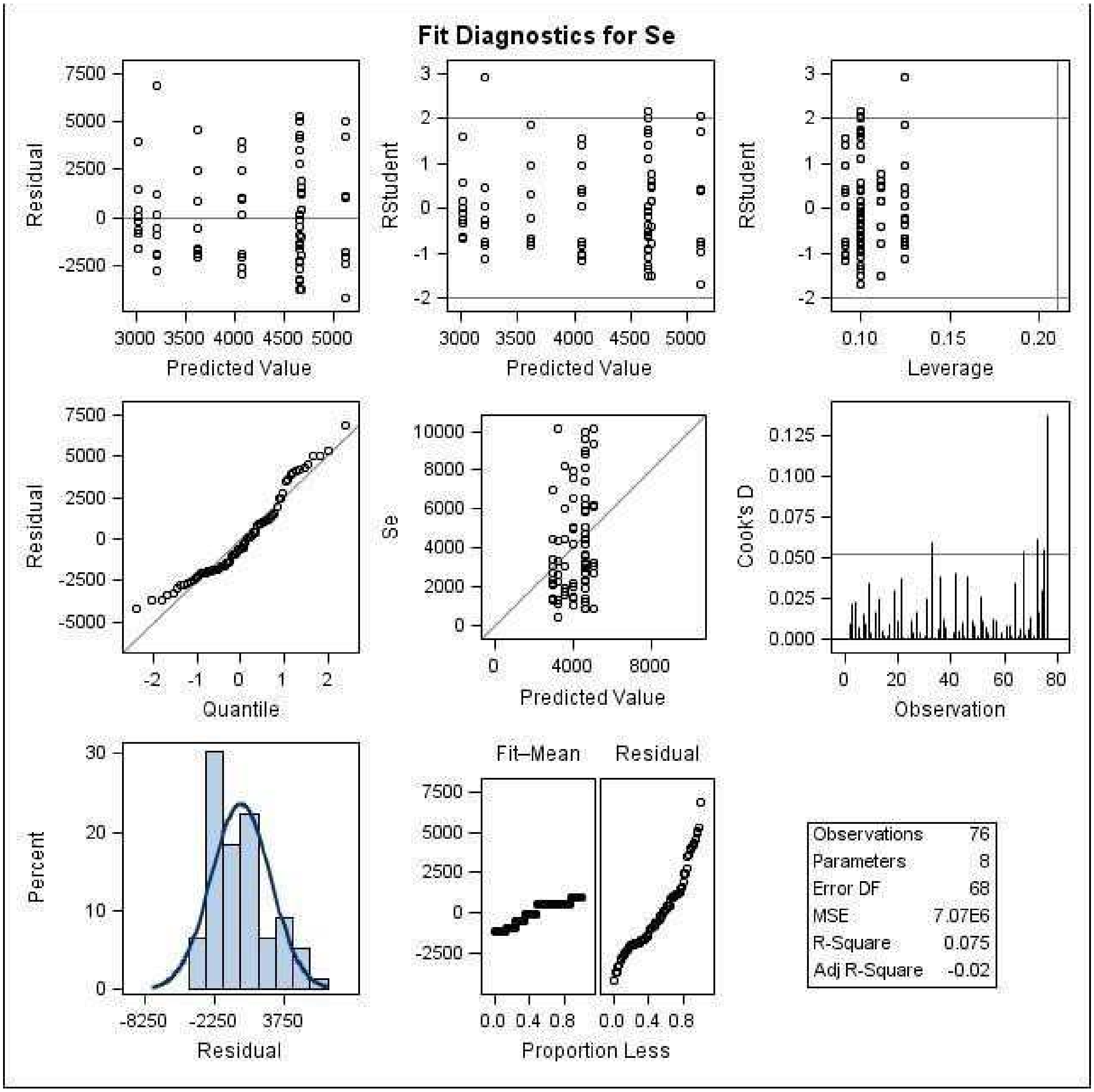
```
0.6856
Light                          1       81194.376       81194.376       0.21
0.6449
Lettuce_Type*Light             1      367956.990      367956.990       0.97
0.3279
Fertilizer*Light               1      729073.198      729073.198       1.92
0.1700
Lettuc*Fertili*Light           1      155286.101      155286.101       0.41
0.5242
```



Dependent Variable: Se

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	255611.25	255611.25	0.04	0.8498
Fertilizer	1	17596804.04	17596804.04	2.49	0.1193
Lettuce_T*Fertilizer	1	1249851.63	1249851.63	0.18	

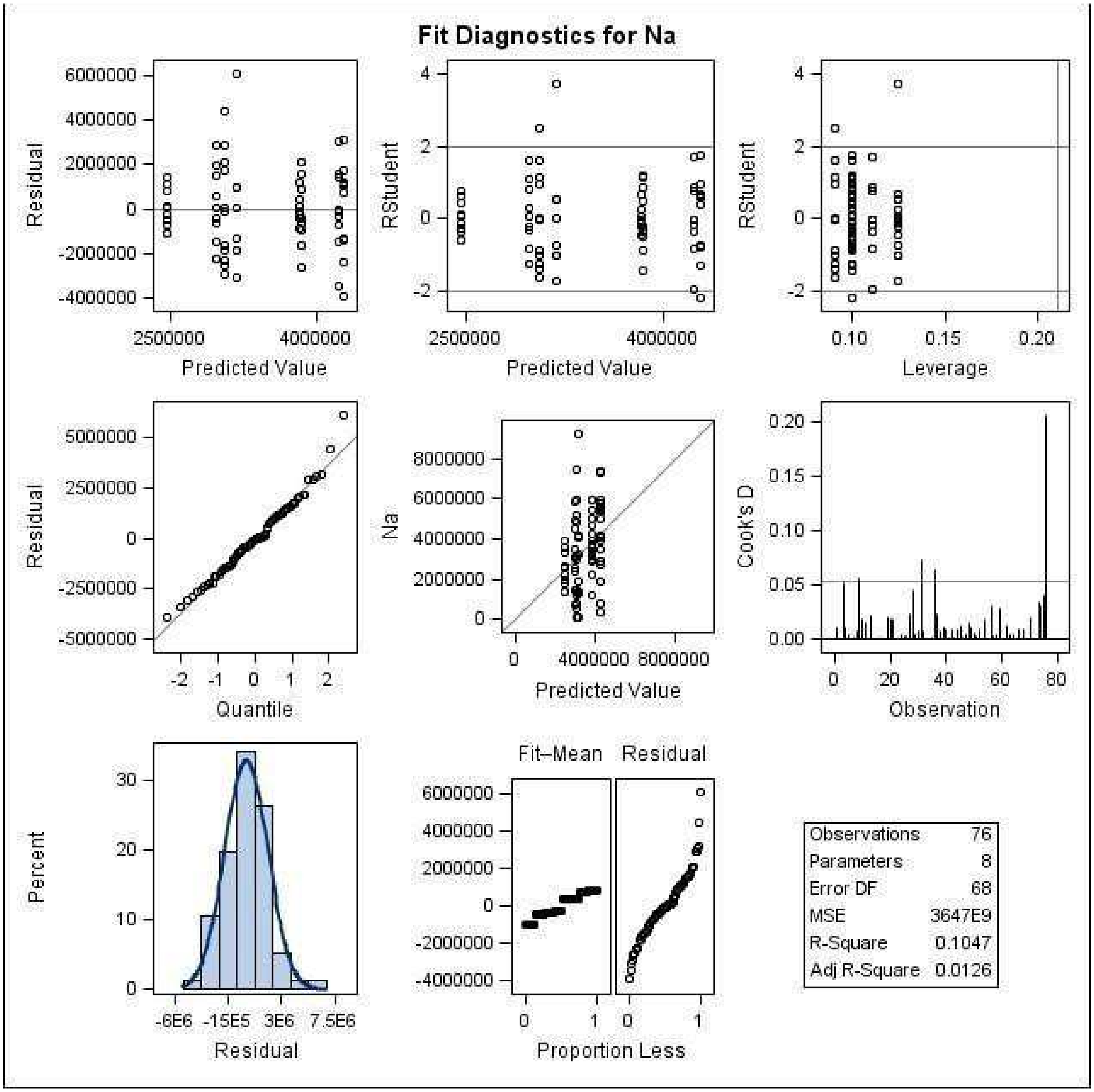
```
0.6755
Light                        1    12204143.15    12204143.15       1.73
0.1934
Lettuce_Type*Light           1     6422782.39     6422782.39       0.91
0.3440
Fertilizer*Light             1     1252136.28     1252136.28
0.18   0.6752
Lettuc*Fertili*Light         1       27126.91       27126.91       0.00
0.9508
```



Dependent Variable: Na

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	359743617392	359743617392	0.10	0.7544
Fertilizer	1	1.6357011E12	1.6357011E12	0.45	0.5053

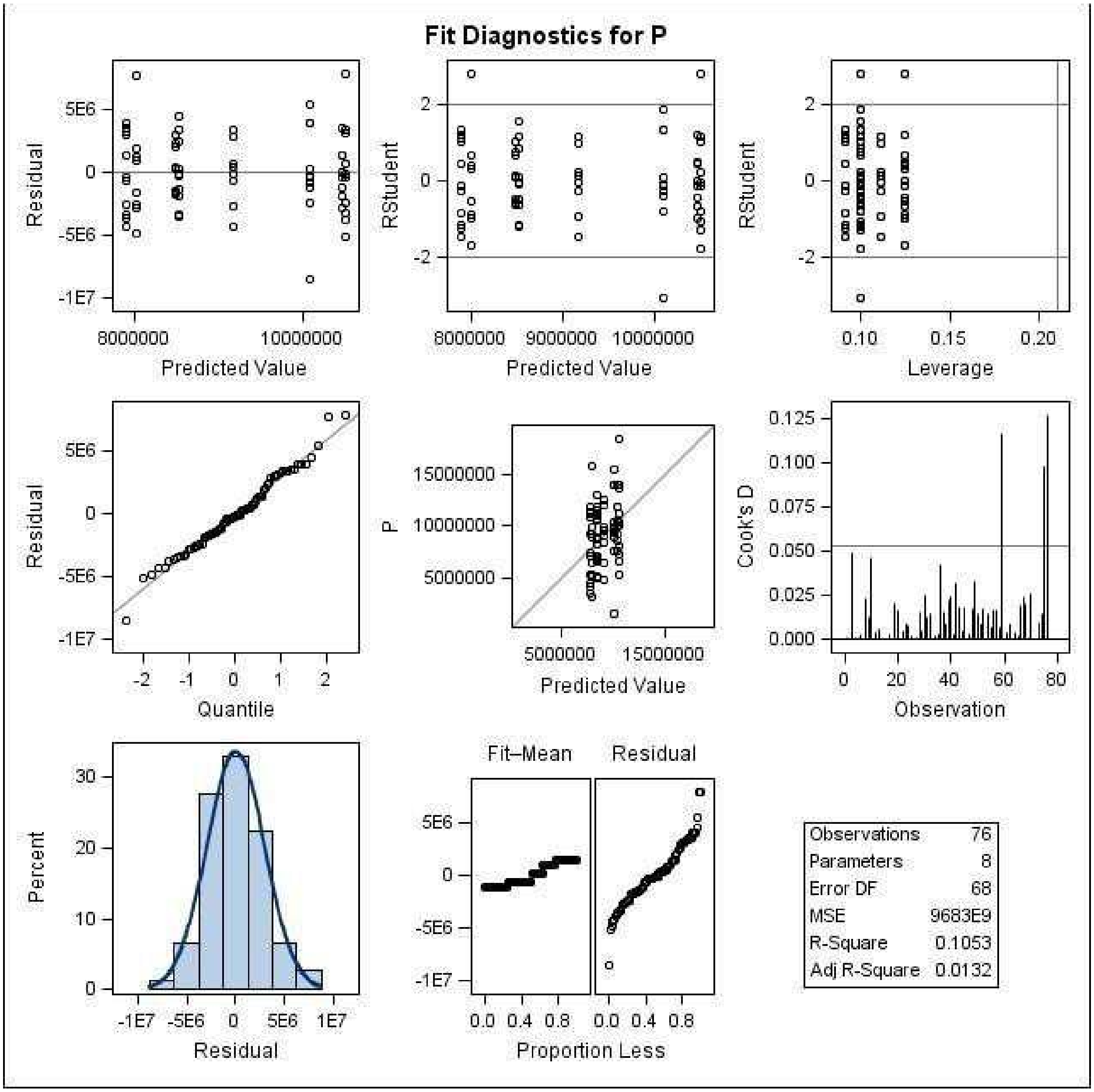
```
Lettuce_T*Fertilizer        1   842921130030   842921130030      0.23
0.6322
Light                       1   5.1413894E12   5.1413894E12      1.41
0.2392
Lettuce_Type*Light          1    1.328962E13    1.328962E13      3.64
0.0605
Fertilizer*Light            1   4.2496283E12   4.2496283E12      1.17
0.2842
Lettuc*Fertili*Light        1   3.4944709E12   3.4944709E12      0.96
0.3311
```



Dependent Variable: P

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	728718102449	728718102449	0.08	0.7847
Fertilizer	1	1.4024901E12	1.4024901E12	0.14	0.7047
Lettuce_T*Fertilizer	1	311575091742	311575091742	0.03	

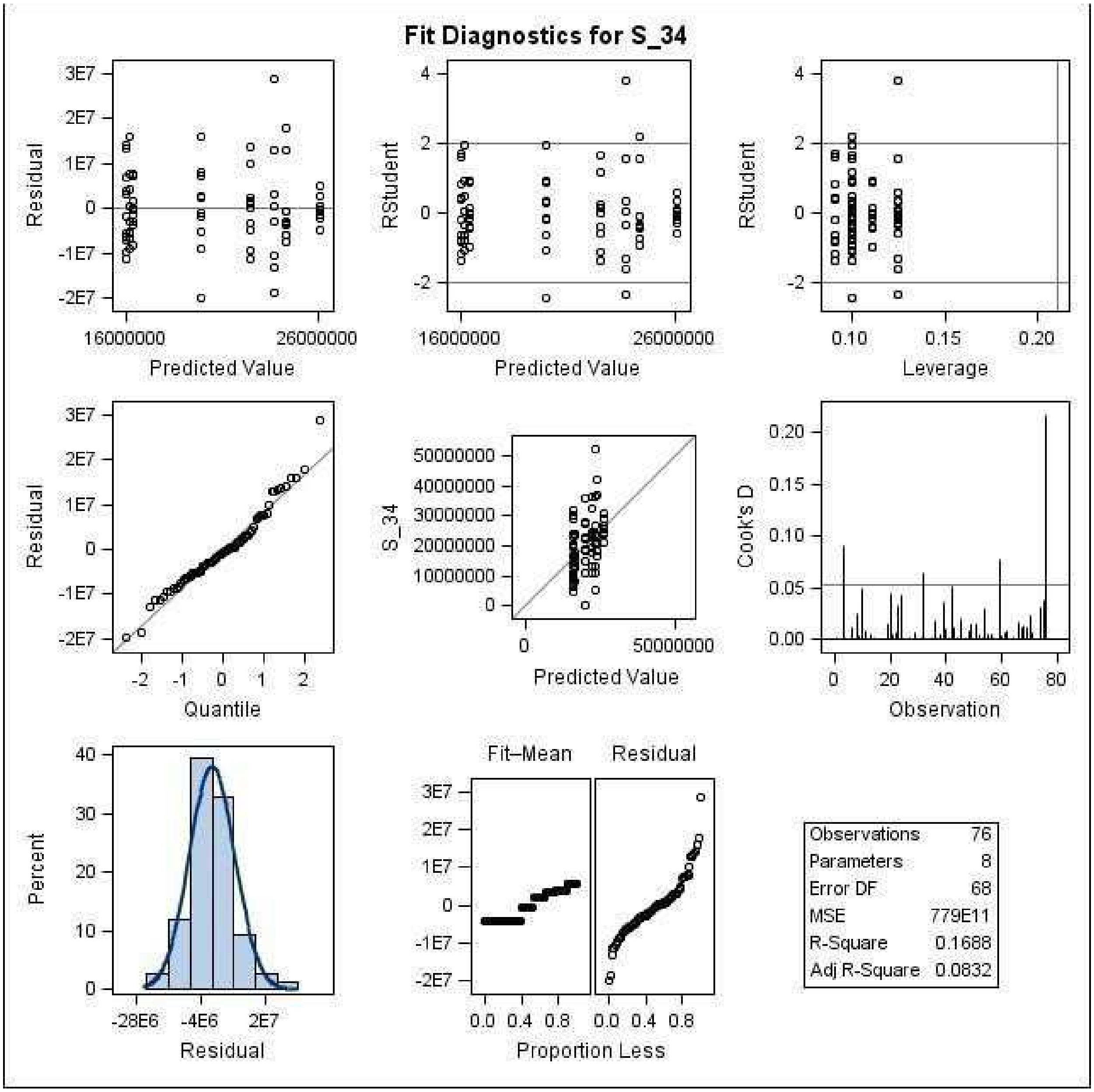
```
0.8582
Light                    1   4.2873572E13   4.2873572E13      4.43
0.0391
Lettuce_Type*Light       1   8.6398967E12   8.6398967E12      0.89
0.3482
Fertilizer*Light         1   3.5888725E12   3.5888725E12      0.37
0.5447
Lettuc*Fertili*Light     1   1.9931661E13   1.9931661E13      2.06
0.1559
```



Dependent Variable: S_34

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	2.5828227E12	2.5828227E12	0.03	0.8560
Fertilizer	1	5.9048266E14	5.9048266E14	7.58	0.0075
Lettuce_T*Fertilizer	1	1.459778E14	1.459778E14	1.88	

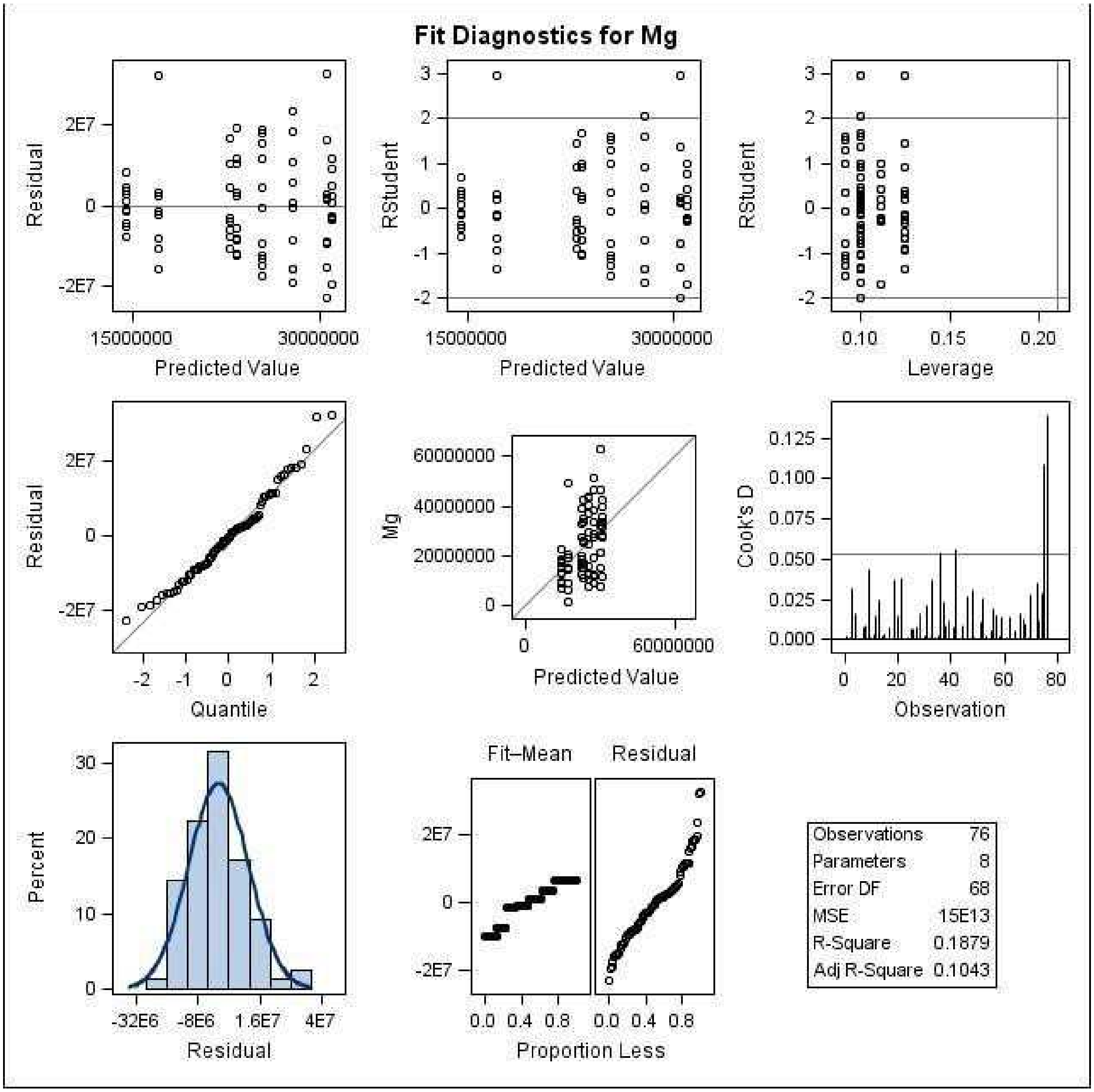
```
0.1754
Light                    1   2.1790202E13   2.1790202E13      0.28
0.5985
Lettuce_Type*Light       1   173687587294   173687587294      0.00
0.9625
Fertilizer*Light         1   1.0101982E14   1.0101982E14
1.30   0.2586
Lettuc*Fertili*Light     1   2.1299886E14   2.1299886E14      2.74
0.1027
```



Dependent Variable: Mg

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	4.7056435E13	4.7056435E13	0.31	0.5778
Fertilizer	1	1.601081E15	1.601081E15	10.64	0.0017
Lettuce_T*Fertilizer	1	816869336590	816869336590	0.01	

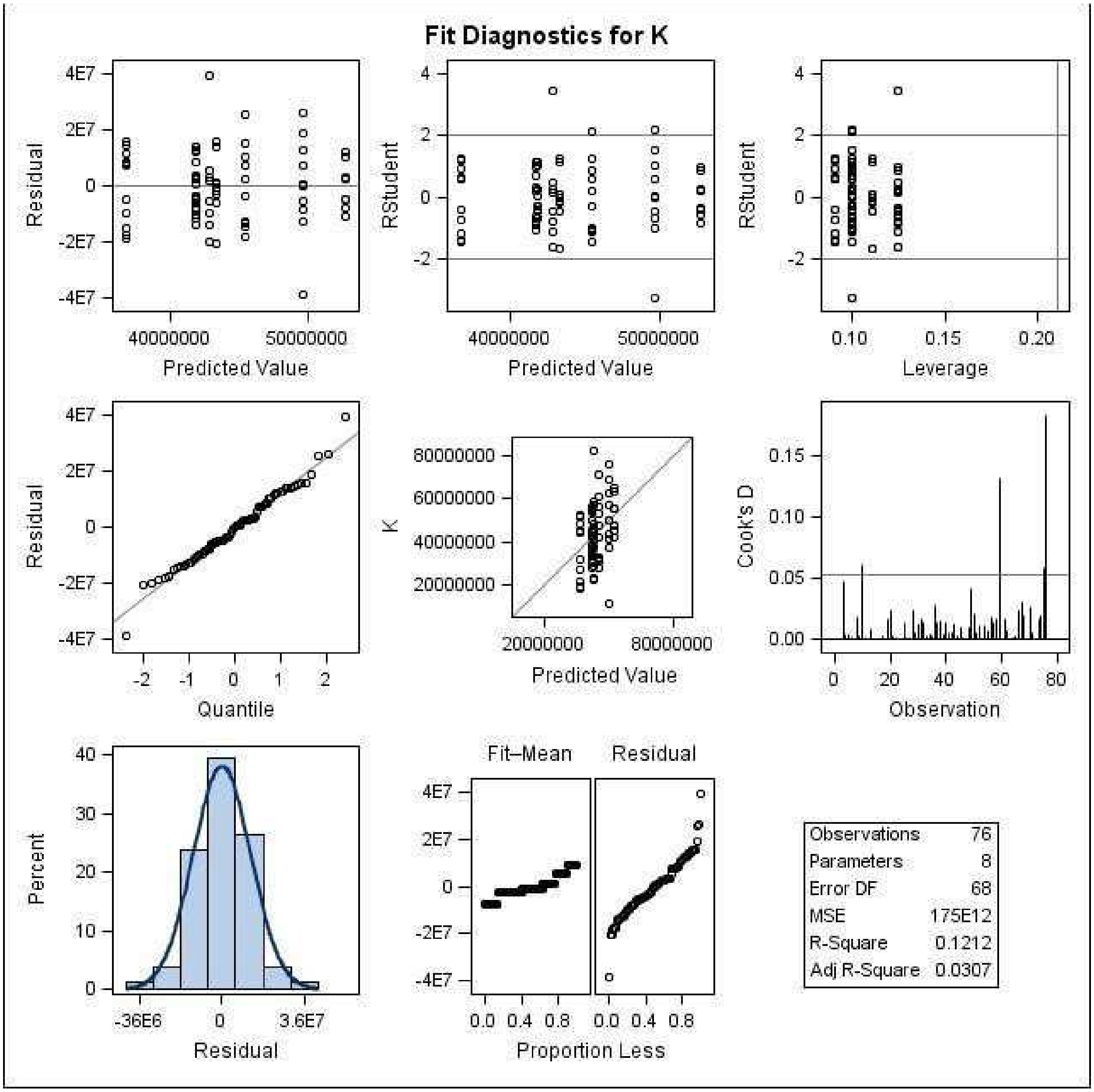
```
0.9415
Light                          1    3.3719504E14    3.3719504E14       2.24
0.1390
Lettuce_Type*Light             1    1.6971494E14    1.6971494E14       1.13
0.2919
Fertilizer*Light               1    1.7879717E14    1.7879717E14
1.19   0.2795
Lettuc*Fertili*Light           1    3.1807722E13    3.1807722E13       0.21
0.6471
```



Dependent Variable: K

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	5.2136672E13	5.2136672E13	0.30	0.5866
Fertilizer	1	4.8428348E14	4.8428348E14	2.77	0.1005
Lettuce_T*Fertilizer	1	843634421896	843634421896	0.00	

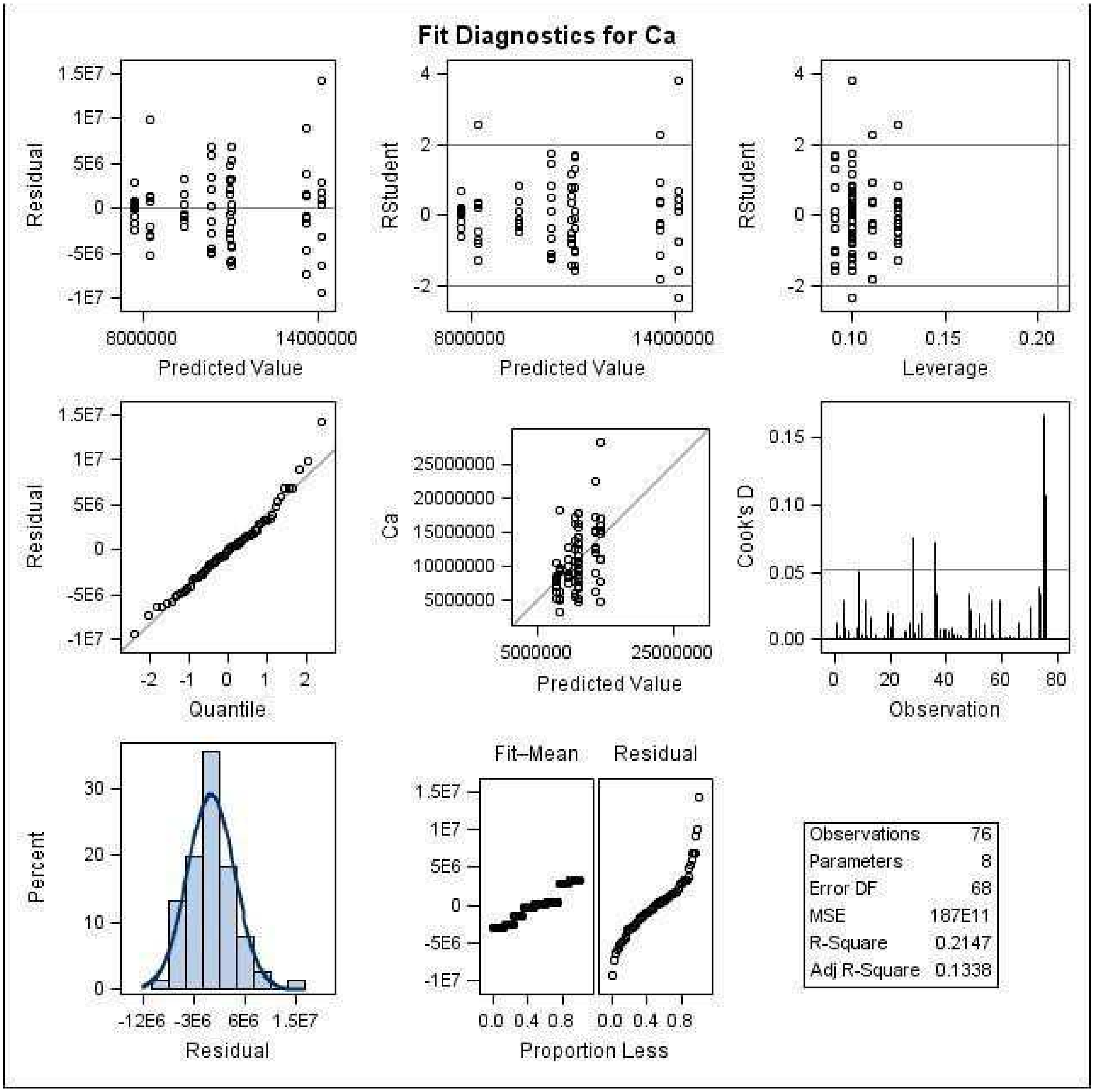
```
0.9448
Light                    1    7.2234586E14   7.2234586E14       4.14
0.0459
Lettuce_Type*Light       1    9.8673475E13   9.8673475E13       0.56
0.4549
Fertilizer*Light         1    1.0606366E14   1.0606366E14
0.61   0.4385
Lettuc*Fertili*Light     1    1.7380356E14   1.7380356E14       0.99
0.3221
```



Dependent Variable: Ca

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	4.4494825E12	4.4494825E12	0.24	0.6271

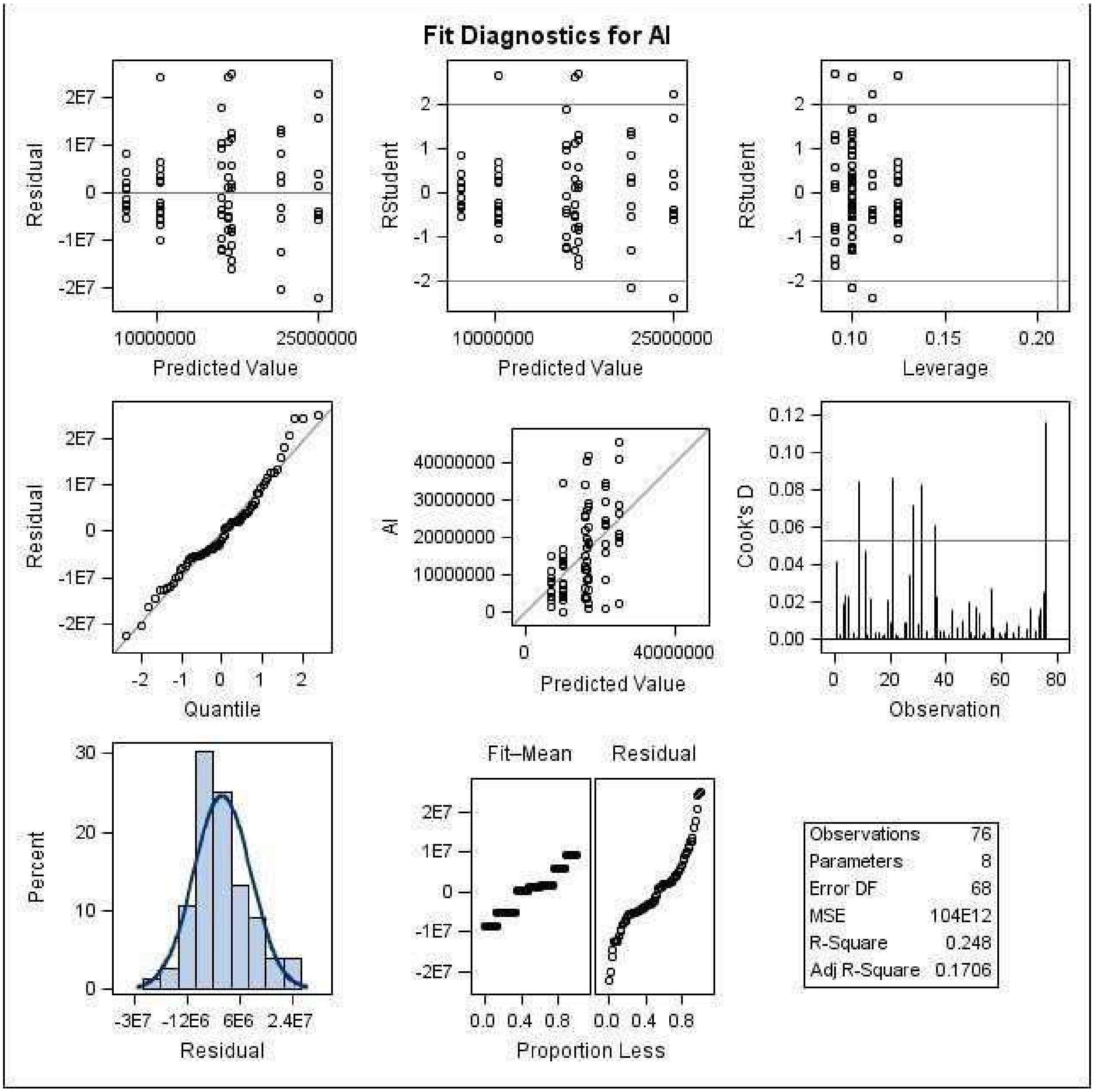
Fertilizer	1	1.8538695E14	1.8538695E14	9.92	0.0024
Lettuce_T*Fertilizer	1	51418817840	51418817840	0.00	0.9583
Light	1	2.5162298E13	2.5162298E13	1.35	0.2499
Lettuce_Type*Light	1	9.0246434E13	9.0246434E13	4.83	0.0314
Fertilizer*Light	1	2.1101892E13	2.1101892E13	1.13	0.2916
Lettuc*Fertili*Light	1	2.0804274E13	2.0804274E13	1.11	0.2950



Dependent Variable: Al

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	270450191406	270450191406	0.00	0.9595
Fertilizer	1	1.3687257E15	1.3687257E15	13.12	0.0006
Lettuce_T*Fertilizer	1	3.3925309E13	3.3925309E13	0.33	

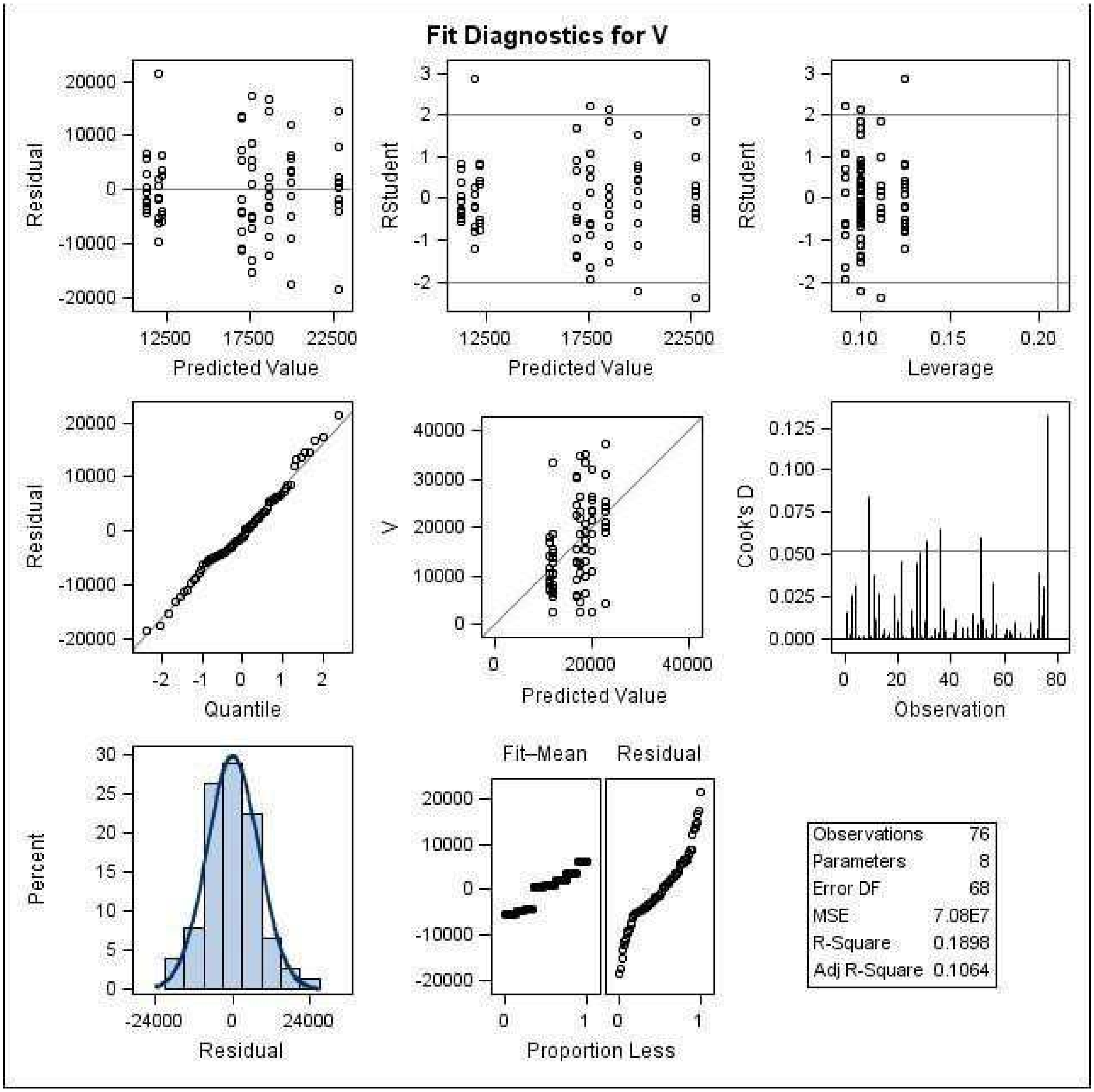
```
0.5704
Light                         1   4.4447491E13   4.4447491E13       0.43
0.5162
Lettuce_Type*Light            1   6.5495414E14   6.5495414E14       6.28
0.0146
Fertilizer*Light              1   2.2035042E14   2.2035042E14
2.11   0.1508
Lettuc*Fertili*Light          1    1.708606E13    1.708606E13       0.16
0.6870
```



Dependent Variable: V

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	7600734.5	7600734.5	0.11	0.7441
Fertilizer	1	591895034.6	591895034.6	8.36	0.0051
Lettuce_T*Fertilizer	1	69512195.7	69512195.7	0.98	

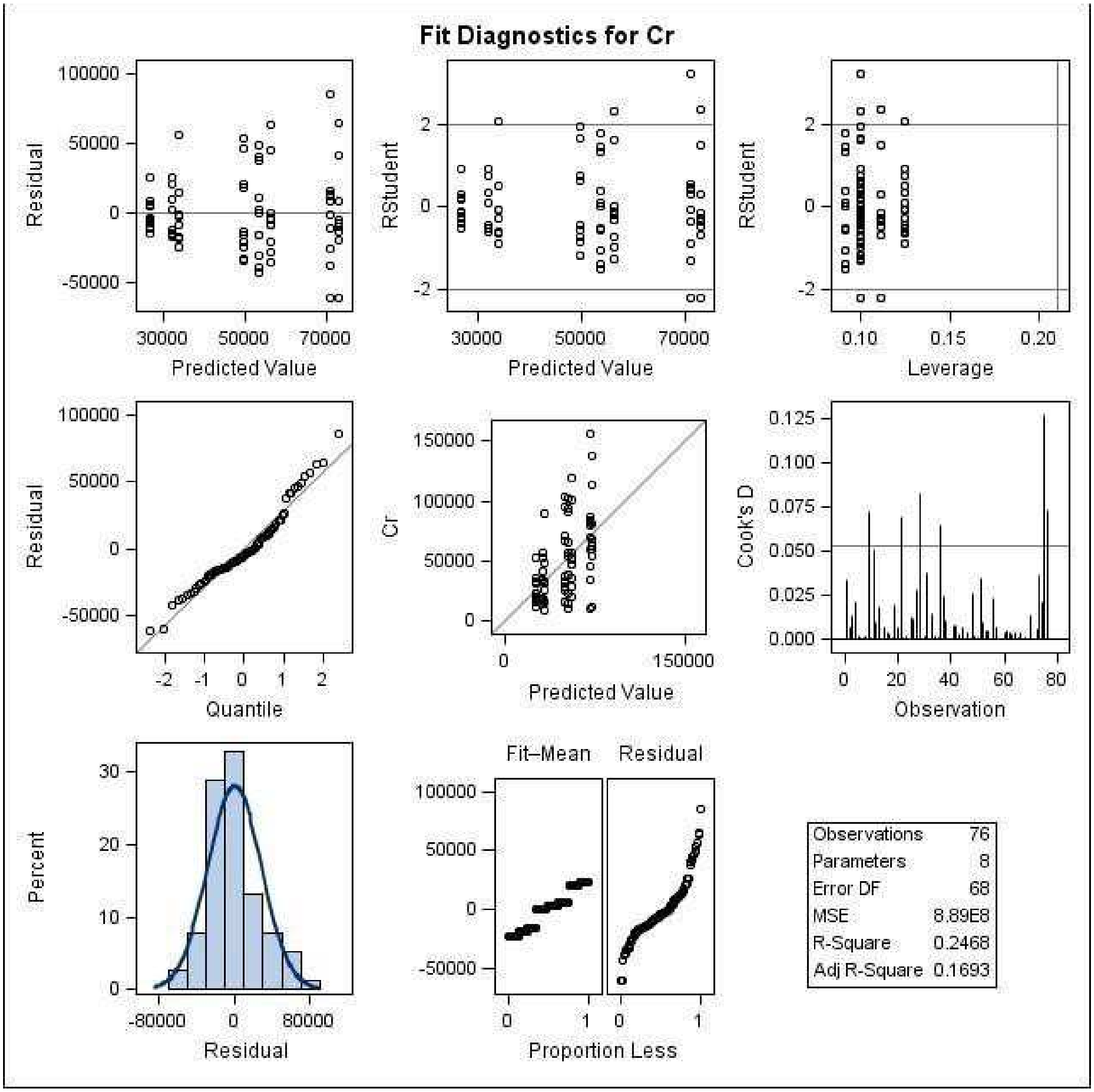
```
0.3251
Light                          1      26230114.0      26230114.0       0.37
0.5447
Lettuce_Type*Light             1     289174726.1     289174726.1       4.09
0.0472
Fertilizer*Light               1     141530165.0     141530165.0
2.00   0.1618
Lettuc*Fertili*Light           1       1344374.8       1344374.8       0.02
0.8908
```



Dependent Variable: Cr

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	285904491	285904491	0.32	0.5725
Fertilizer	1	10733422155	10733422155	12.07	0.0009
Lettuce_T*Fertilizer	1	250198607	250198607	0.28	

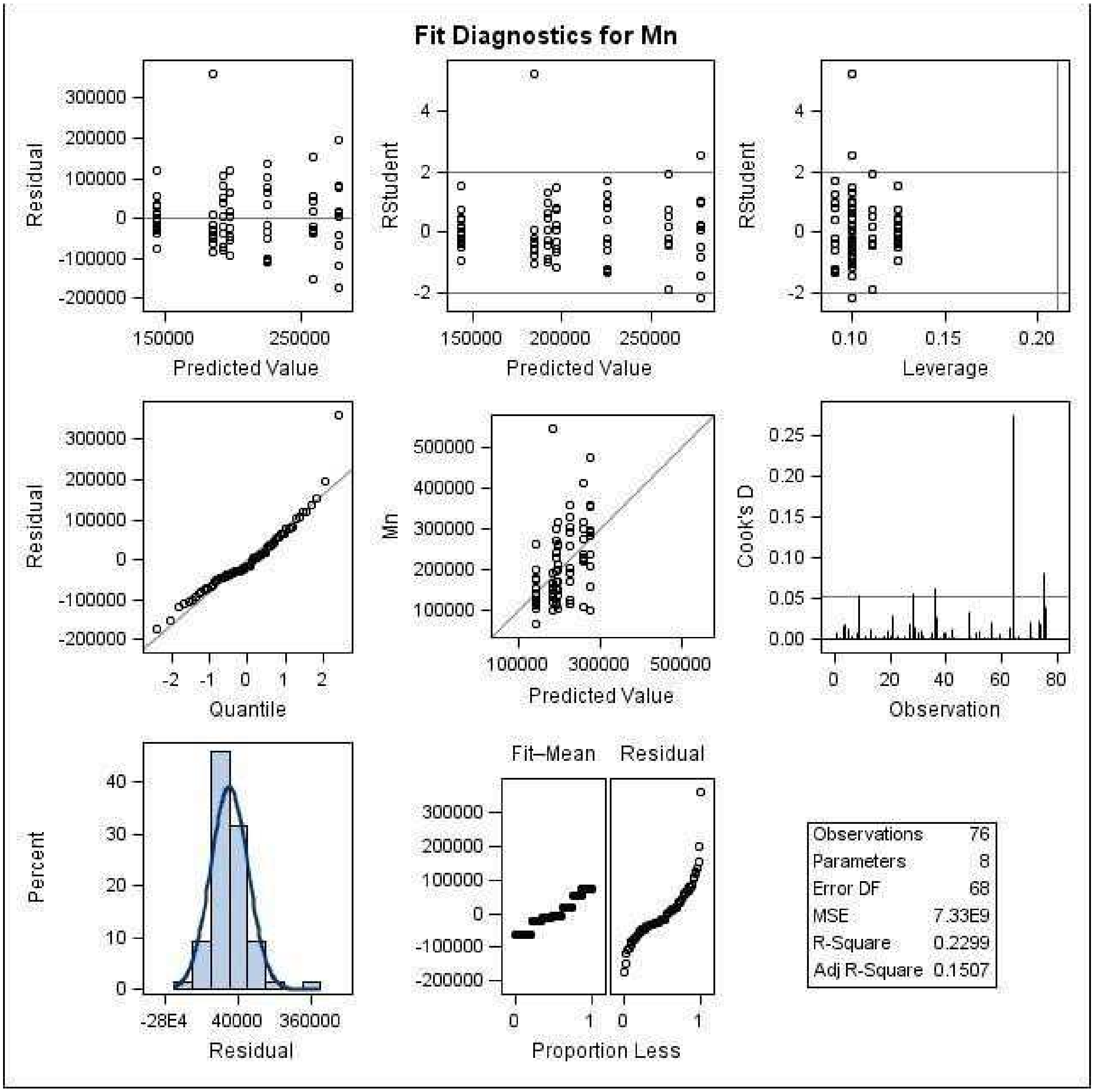
					0.5975
Light	1	758947826	758947826	0.85	0.3587
Lettuce_Type*Light	1	6382723879	6382723879	7.18	0.0092
Fertilizer*Light	1	1294298077	1294298077	1.46	0.2317
Lettuc*Fertili*Light	1	104997723	104997723	0.12	0.7321



Dependent Variable: Mn

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	21555579397	21555579397	2.94	0.0910
Fertilizer	1	89190422879	89190422879	12.16	0.0009
Lettuce_T*Fertilizer	1	2043090477	2043090477	0.28	

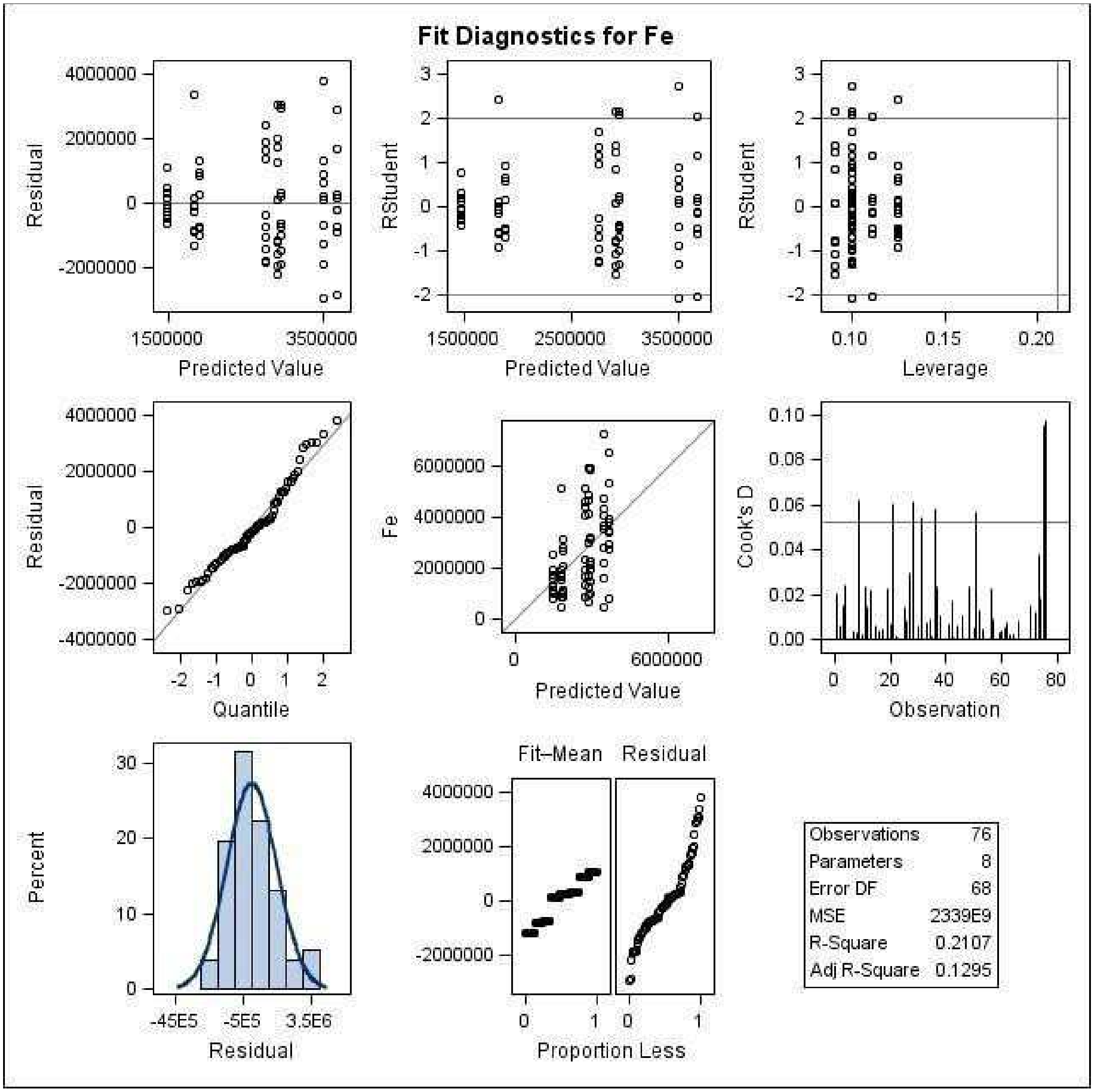
```
0.5993
Light                       1       24568277       24568277       0.00
0.9540
Lettuce_Type*Light          1    22233031639    22233031639       3.03
0.0861
Fertilizer*Light            1      412136428      412136428
0.06   0.8133
Lettuc*Fertili*Light        1    13411114724    13411114724       1.83
0.1807
```



Dependent Variable: Fe

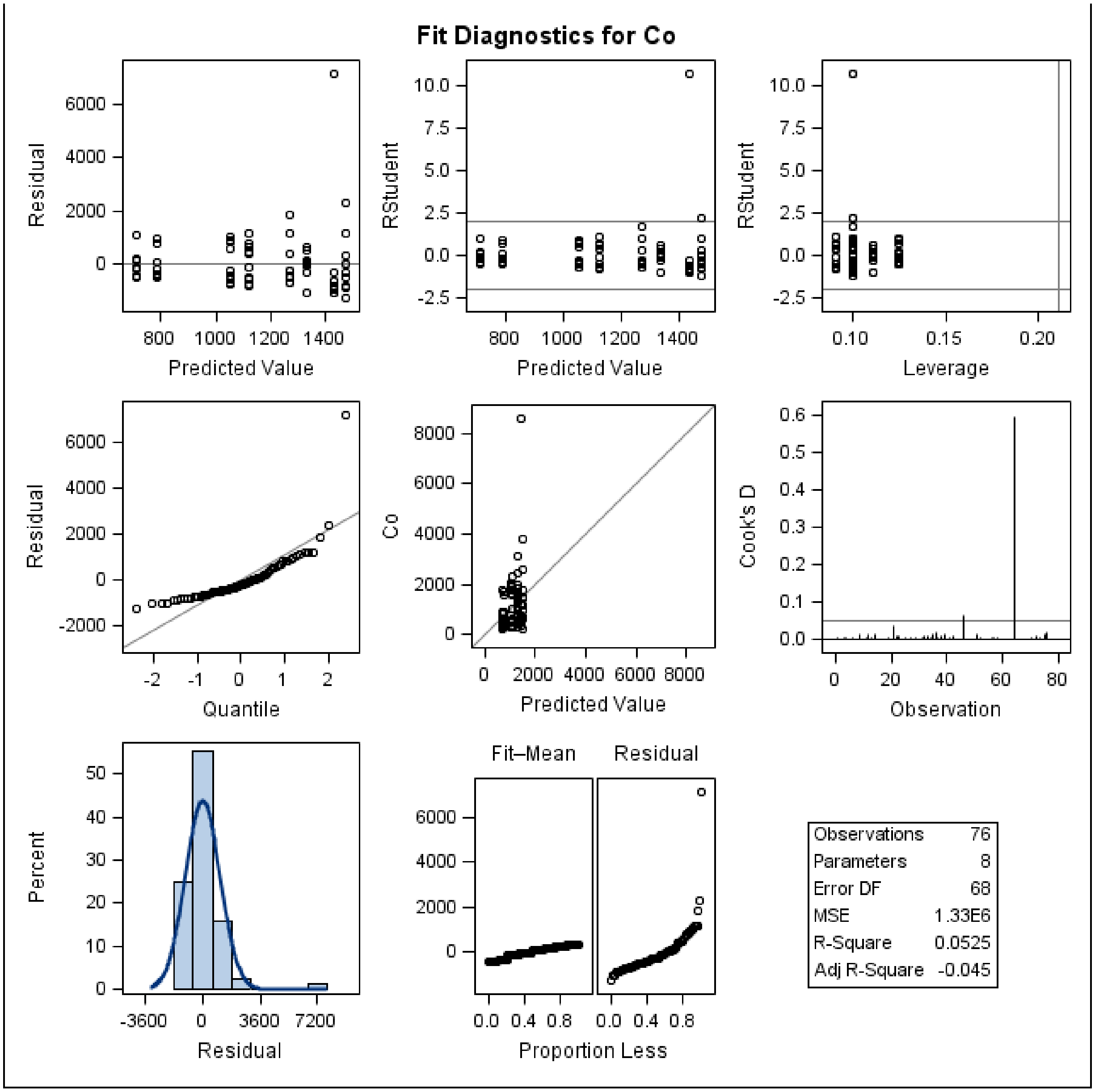
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	334664914512	334664914512	0.14	0.7064
Fertilizer	1	2.4685492E13	2.4685492E13	10.55	0.0018
Lettuce_T*Fertilizer	1	583689354180	583689354180	0.25	0.6190
Light	1	2.1225421E12	2.1225421E12	0.91	0.3441
Lettuce_Type*Light	1	1.0587456E13	1.0587456E13	4.53	0.0370
Fertilizer*Light	1	4.1385889E12	4.1385889E12	1.77	0.1879
Lettuc*Fertili*Light	1	9553930188.3			

9553930188.3 0.00 0.9492



Source	DF	Type I SS	Mean Square	F Value	Pr > F
Dependent Variable: Co					
Lettuce_Type	1	2109841.986	2109841.986	1.59	0.2115
Fertilizer	1	526411.689	526411.689	0.40	0.5308
Lettuce_T*Fertilizer	1	1174207.253	1174207.253	0.89	0.3500
Light	1	79.589	79.589	0.00	0.9938
Lettuce_Type*Light	1	228310.166	228310.166	0.17	0.6795
Fertilizer*Light	1	60280.352	60280.352	0.05	0.8318
Lettuc*Fertili*Light	1	901705.798	901705.798	0.68	0.4125

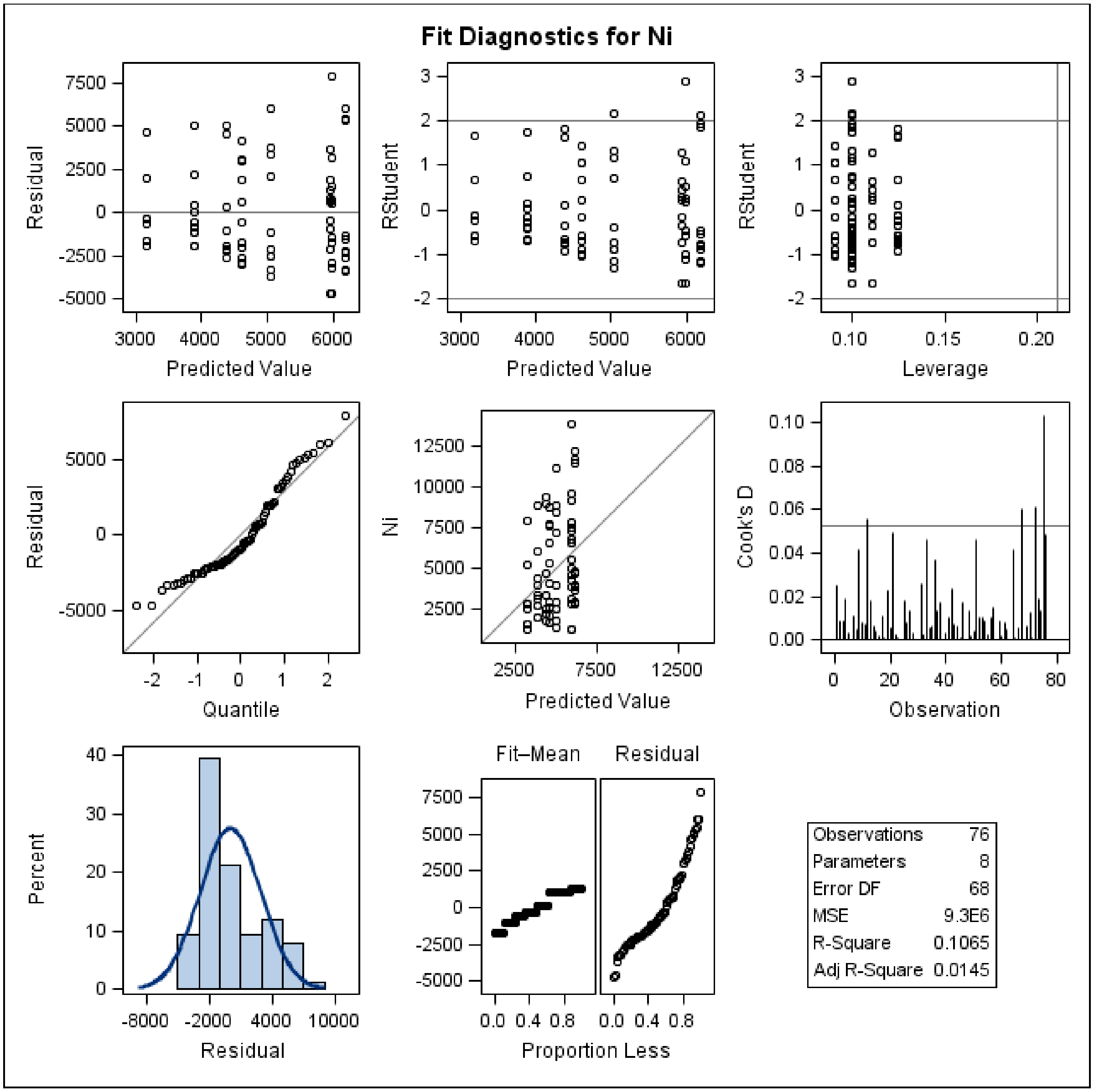
Source DF Type I SS Mean Square F Value Pr > F



Dependent Variable: Ni

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	4014825.54	4014825.54	0.43	0.5134
Fertilizer	1	15403959.13	15403959.13	1.66	0.2025
Lettuce_T*Fertilizer	1	10226698.11	10226698.11	1.10	0.2981
Light	1	19354575.33	19354575.33	2.08	0.1538
Lettuce_Type*Light	1	14621616.88	14621616.88	1.57	0.2142
Fertilizer*Light	1	10162814.49	10162814.49	1.09	0.2996
Lettuc*Fertili*Light	1	1598664.08	1598664.08	0.17	0.6798

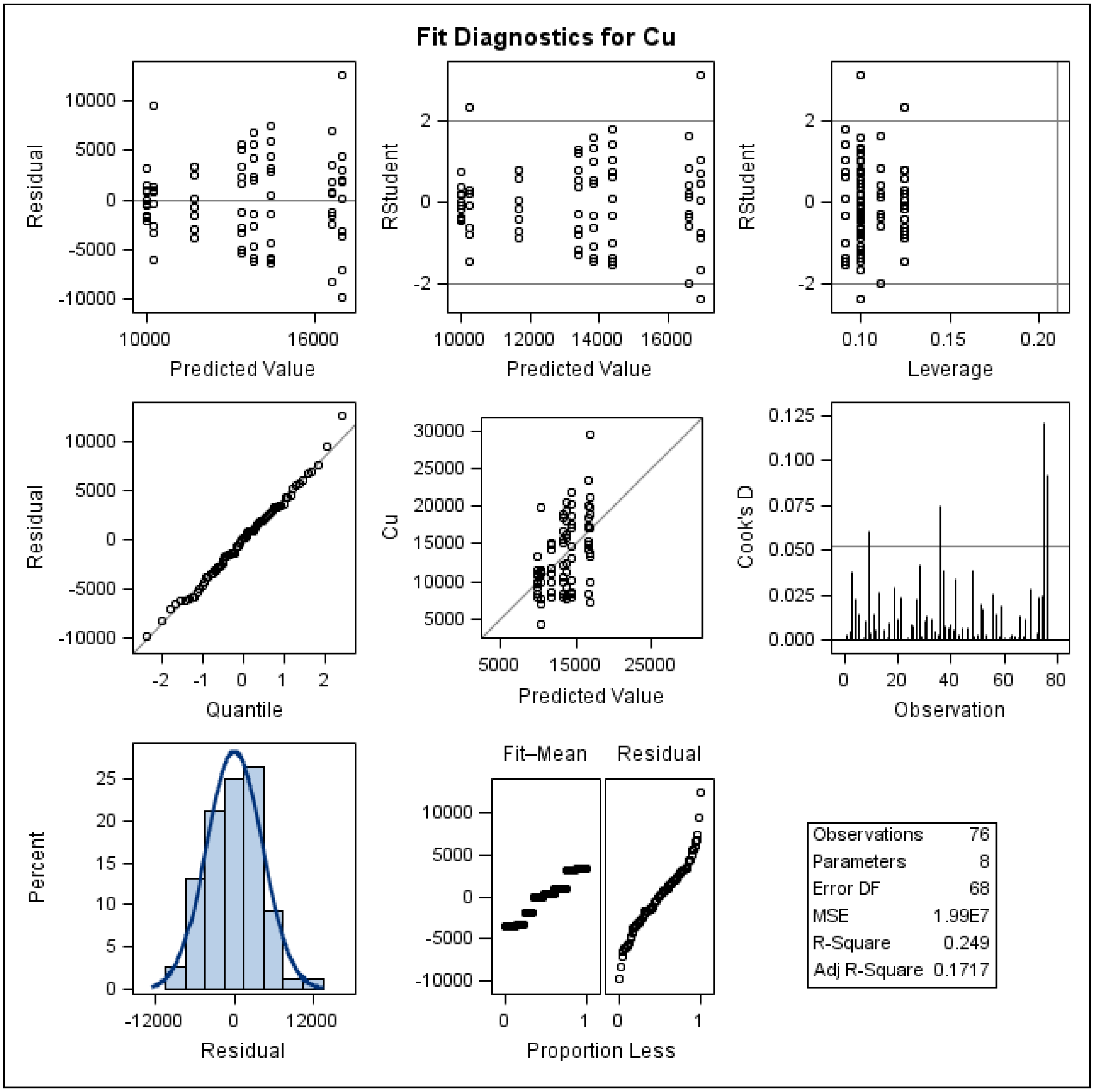
Source DF Type I SS Mean Square F Value Pr > F



Dependent Variable: Cu

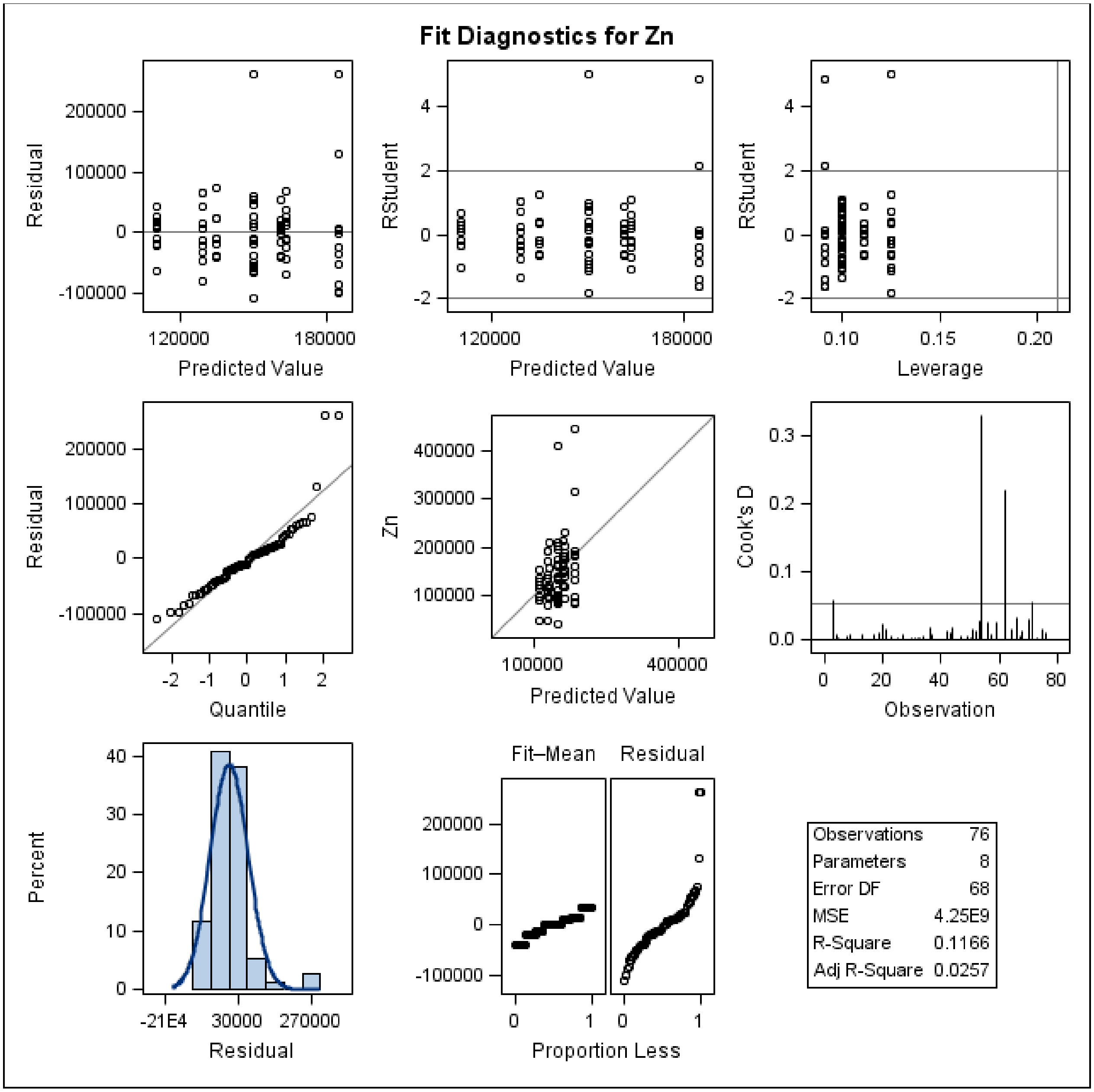
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	4069610.9	4069610.9	0.20	0.6524
Fertilizer	1	308516846.0	308516846.0	15.52	0.0002
Lettuce_T*Fertilizer	1	288579.8	288579.8	0.01	0.9045
Light	1	27739605.7	27739605.7	1.40	0.2417
Lettuce_Type*Light	1	68336328.4	68336328.4	3.44	0.0681
Fertilizer*Light	1	26326714.7	26326714.7	1.32	0.2539
Lettuc*Fertili*Light	1	13026917.4	13026917.4	0.66	0.4211

Source DF Type I SS Mean Square F Value
Pr > F



Dependent Variable: Zn

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	50200866	50200866	0.01	0.9138
Fertilizer	1	24144812473	24144812473	5.68	0.0200
Lettuce_T*Fertilizer	1	8252037712	8252037712	1.94	0.1682
Light	1	921533561	921533561	0.22	0.6431
Lettuce_Type*Light	1	618326548	618326548	0.15	0.7042
Fertilizer*Light	1	1875479788	1875479788	0.44	0.5089
Lettuc*Fertili*Light	1	2308126078	2308126078	0.54	0.4638



Dependent Variable: Sr Source DF Type I SS

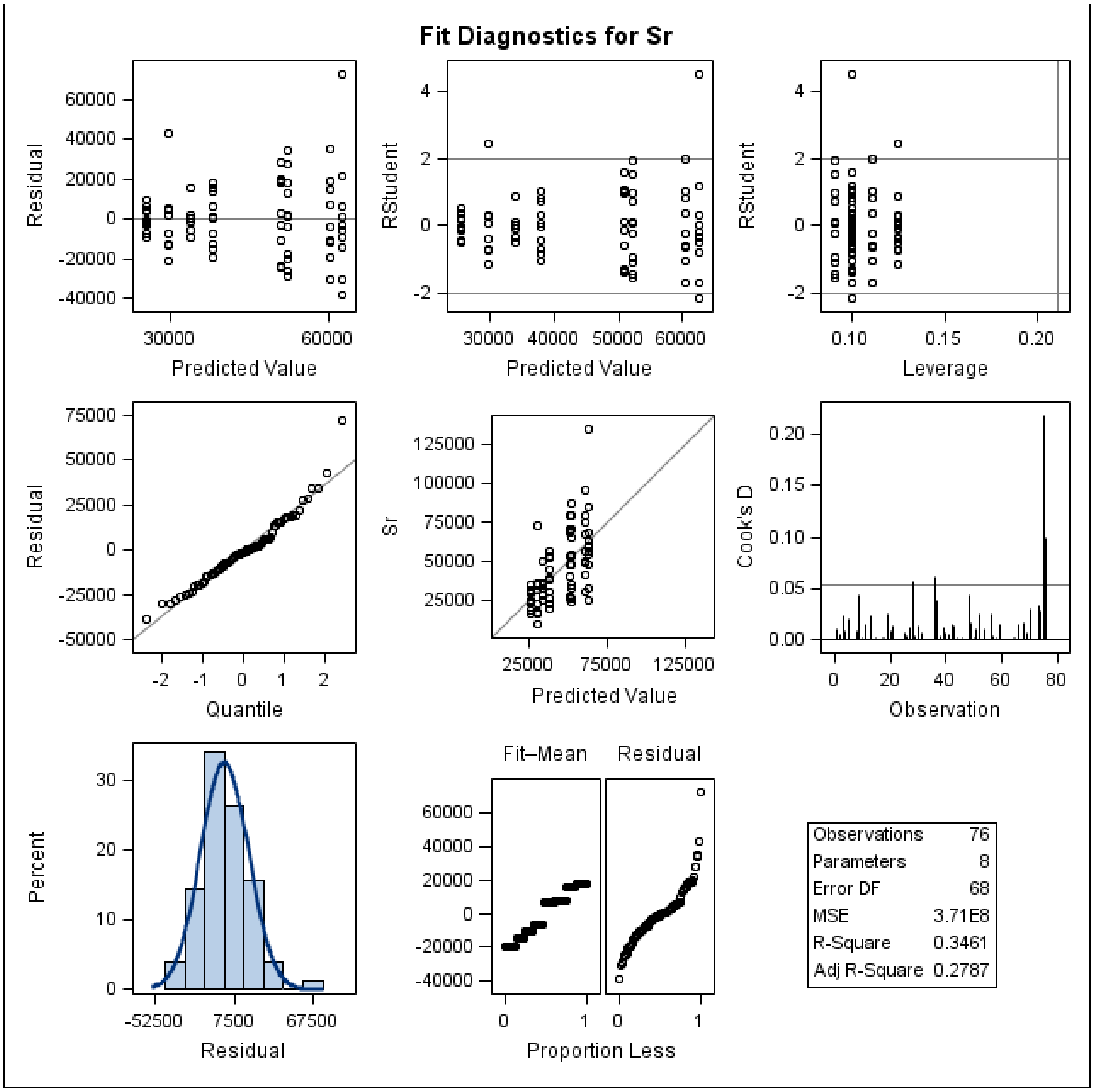
Mean Square F Value

Pr > F

Lettuce_Type 1 149557 149557 0.00

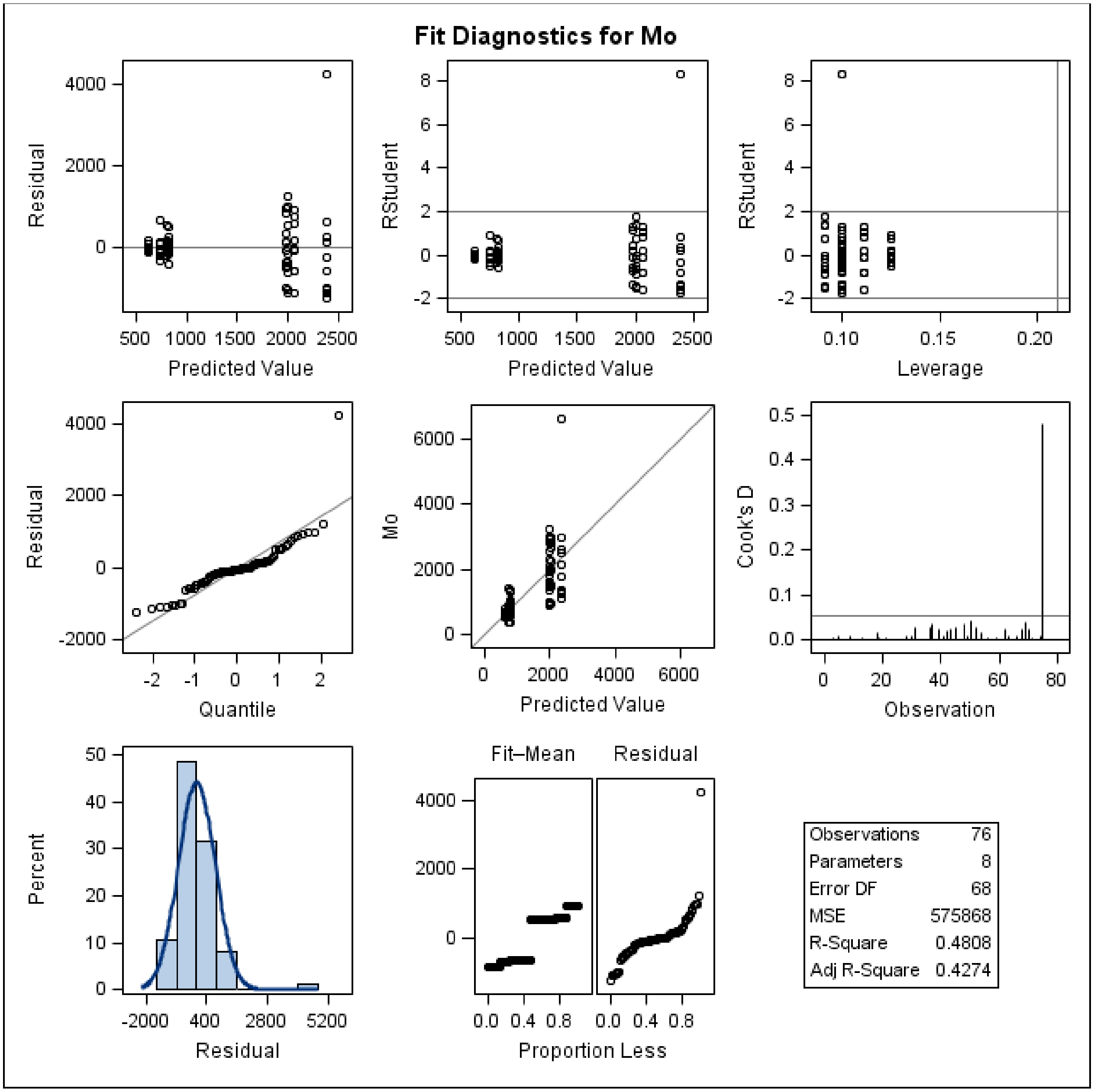
0.9840

Fertilizer	1	11499611887	11499611887	30.98	<.0001
Lettuce_T*Fertilizer	1	20326436	20326436	0.05	0.8157
Light	1	408462813	408462813	1.10	0.2979
Lettuce_Type*Light	1	1008384782	1008384782	2.72	0.1039
Fertilizer*Light	1	266939155	266939155	0.72	0.3994
Lettuc*Fertili*Light	1	152274662	152274662	0.41	0.5240


Fit Diagnostics for Sr
Residual
Predicted Value
RStudent
Leverage
Quantile
Sr
Cook's D
Observation
Percent
Fit–Mean
Residual
Proportion Less
Observations 76
Parameters 8
Error DF 68
MSE 3.71E8
R-Square 0.3461
Adj R-Square 0.2787

Dependent Variable: Mo

Source	**DF**	**Type I SS**	**Mean Square**	**F Value**	**Pr > F**
Lettuce_Type	1	5458.90	5458.90	0.01	0.9227
Fertilizer	1	35025859.96	35025859.96	60.82	<.0001
Lettuce_T*Fertilizer	1	242314.58	242314.58	0.42	0.5187
Light	1	428486.22	428486.22	0.74	0.3914
Lettuce_Type*Light	1	441574.72	441574.72	0.77	0.3843
Fertilizer*Light	1	1923.44	1923.44	0.00	0.9541
Lettuc*Fertili*Light	1	122567.83	122567.83	0.21	0.6460



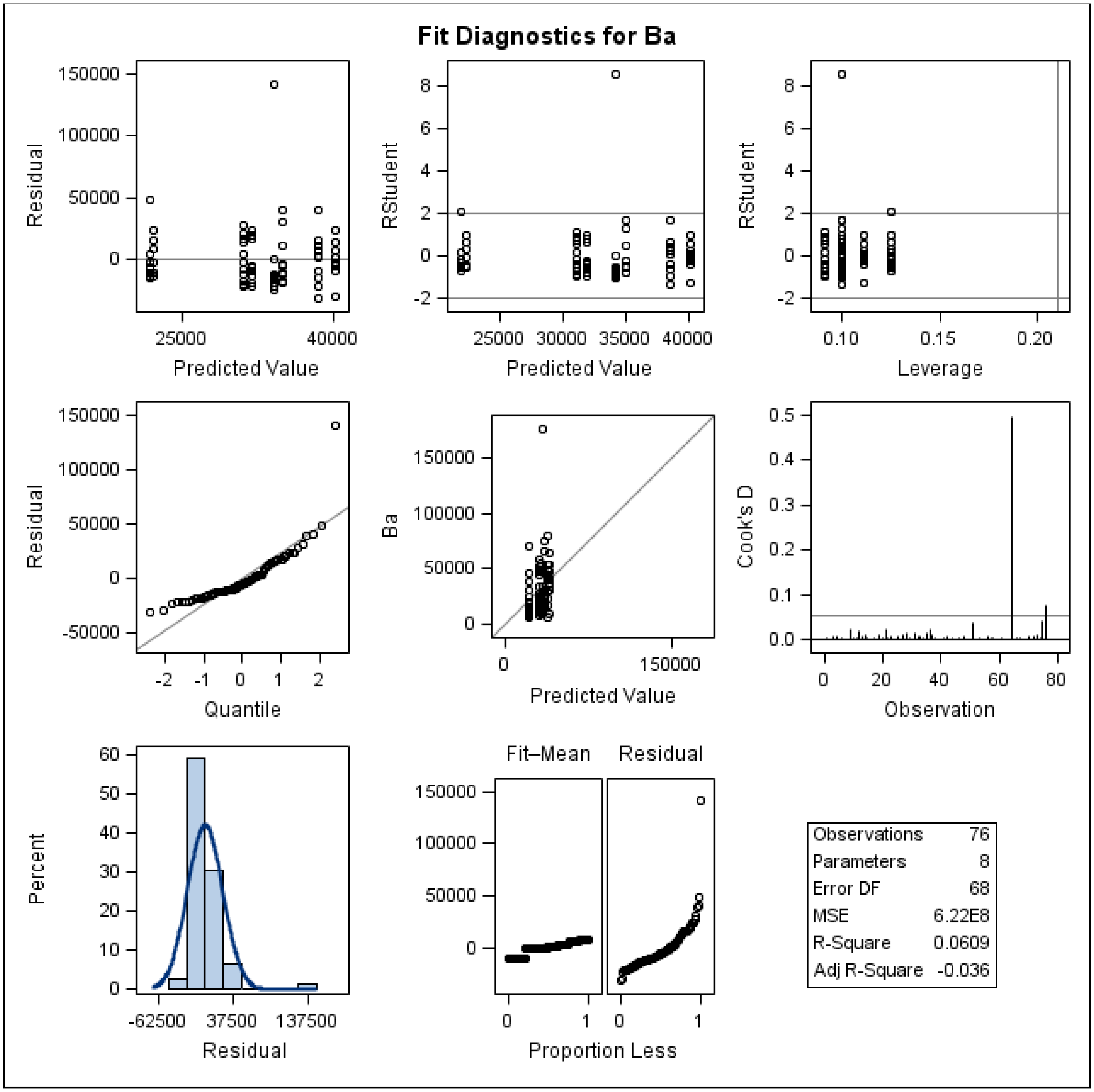
Dependent Variable: Ba Source DF Type I SS

Mean Square F Value

Pr > F

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	481028557.6	481028557.6	0.77	0.3822
Fertilizer	1	758239027.9	758239027.9	1.22	0.2734

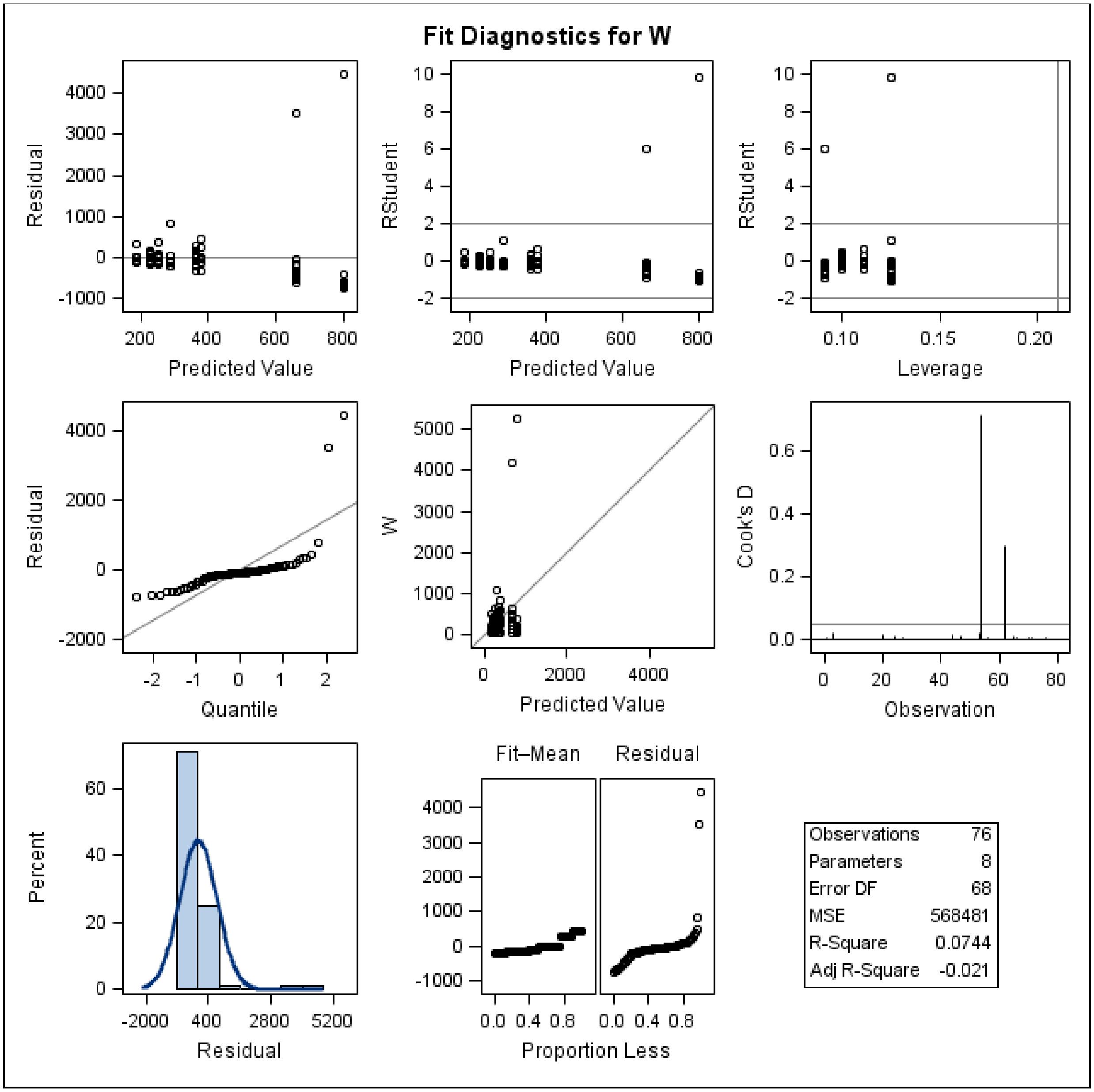
Lettuce_T*Fertilizer	1	886317511.8	886317511.8	1.43	0.2367
Light	1	1934035.1	1934035.1	0.00	0.9557
Lettuce_Type*Light	1	342186653.4	342186653.4	0.55	0.4607
Fertilizer*Light	1	657526.4	657526.4	0.00	0.9742
Lettuc*Fertili*Light	1	271493331.0	271493331.0	0.44	0.5110



Observations	76
Parameters	8
Error DF	68
MSE	6.22E8
R-Square	0.0609
Adj R-Square	-0.036

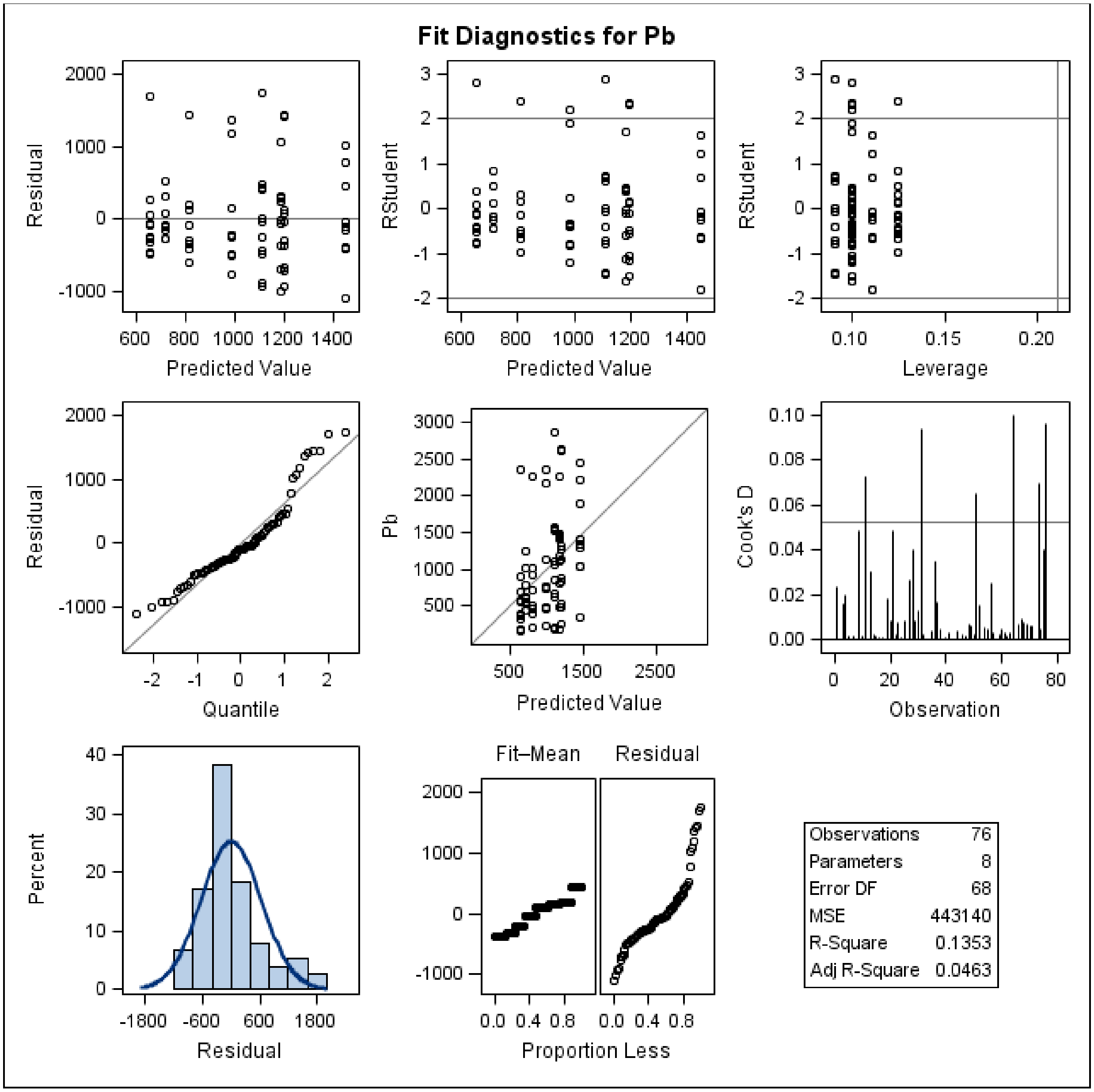
Dependent Variable: W

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	26754.168	26754.168	0.05	0.8289
Fertilizer	1	45587.952	45587.952	0.08	0.7779
Lettuce_T*Fertilizer	1	1386721.094	1386721.094	2.44	0.1230
Light	1	840423.815	840423.815	1.48	0.2282
Lettuce_Type*Light	1	185203.043	185203.043	0.33	0.5700
Fertilizer*Light	1	5878.577	5878.577	0.01	0.9193
Lettuc*Fertili*Light	1	615749.458	615749.458	1.08	0.3017


Fit Diagnostics for W
Residual
Predicted Value
RStudent
Leverage
Quantile
W
Cook's D
Observation
Percent
Residual
Fit–Mean
Proportion Less
Observations 76
Parameters 8
Error DF 68
MSE 568481
R-Square 0.0744
Adj R-Square -0.021

Dependent Variable: Pb

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	110508.890	110508.890	0.25	0.6191
Fertilizer	1	3460445.493	3460445.493	7.81	0.0067
Lettuce_T*Fertilizer	1	238344.220	238344.220	0.54	0.4658
Light	1	11133.879	11133.879	0.03	0.8745
Lettuce_Type*Light	1	670255.343	670255.343	1.51	0.2230
Fertilizer*Light	1	210484.964	210484.964	0.47	0.4930
Lettuc*Fertili*Light	1	13047.342	13047.342	0.03	0.8643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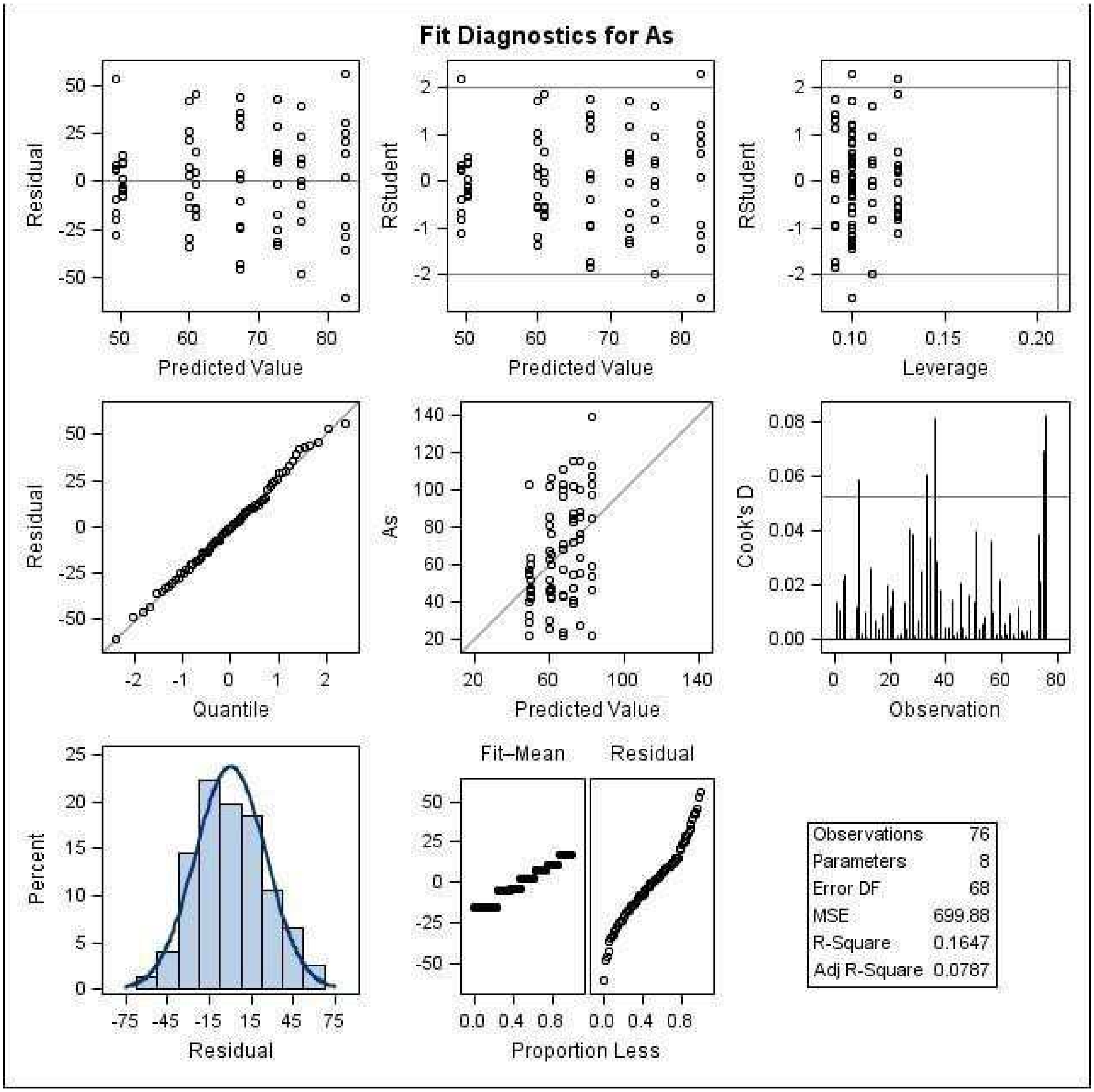
Predicted Value
RStudent
Predicted Value
RStudent
Leverage
Residual
Quantile
Pb
Predicted Value
Cook's D
Observation
Percent
Residual
Fit–Mean
Residual
Proportion Less
Observations 76
Parameters 8
Error DF 68
MSE 443140
R-Square 0.1353
Adj R-Square 0.0463

Dependent Variable: As

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Lettuce_Type	1	736.373045	736.373045	1.05	0.3086
Fertilizer	1	3098.054858	3098.054858	4.43	0.0391
Lettuce_T*Fertilizer	1	1.448162	1.448162	0.00	0.9639
Light	1	1368.159749	1368.159749	1.95	0.1666
Lettuce_Type*Light	1	2348.914000	2348.914000	3.36	0.0713
Fertilizer*Light	1	1310.966289	1310.966289	1.87	0.1756
Lettuc*Fertili*Light	1	521.404628	521.404628	0.74	0.3911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Predicted Value
RStudent
Predicted Value
RStudent
Leverage
Residual
Quantile
As
Predicted Value
Cook's D
Observation
Percent
Residual
Fit–Mean
Residual
Proportion Less
Observations 76
Parameters 8
Error DF 68
MSE 699.88
R-Square 0.1647
Adj R-Square 0.0787

APPENDIX (C) THE FIRST TWO PRINCIPAL COMPONENTS SCORES & DIAGNOSTIC PLOTS FROM ANOVA

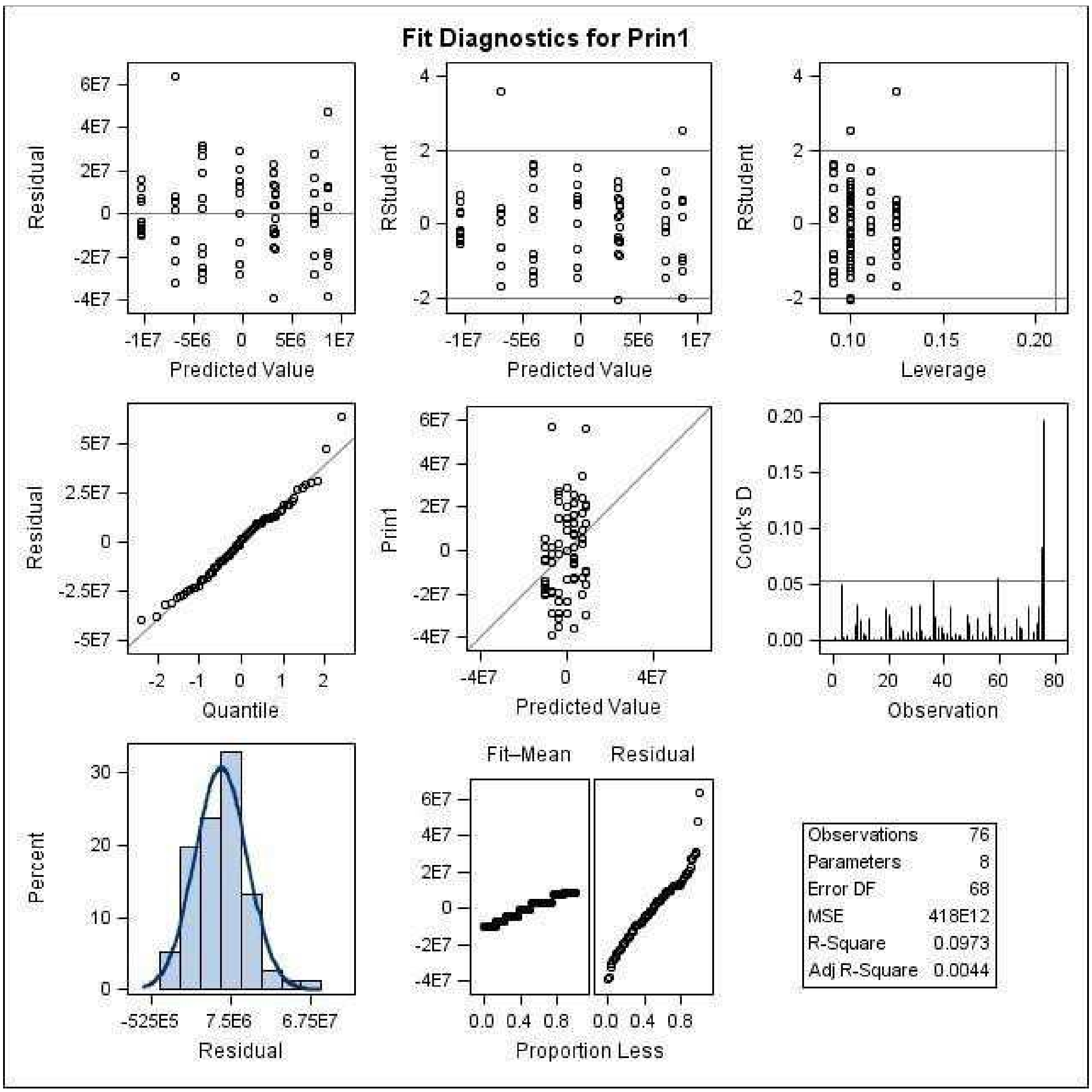
Obs	Analyte	Lettuce_Type	Fertilizer	Light	Prin1	Prin2
1	L28-S13-	Gourmet	NON	LED	16810106.68	-22713825.53
2	L67-S4-G	Gourmet	NON	LED	2789883.44	-6972791.86
3	L09-O21-	Gourmet	Organic	LED	-39060594.19	-2869067.77
4	L55-O51-	Green	Organic	NO	-5812134.45	19852056.64
5	L31-S50-	Green	NON	NO	20616499.46	-6376849.19
6	L42-O3-G	Green	Organic	LED	-15620738.34	3222576.52
7	L54-O39-	Green	Organic	NO	-3811176.11	8088231.22
8	L33-S42-	Green	NON	NO	-10536077.03	-12450205.25
9	L29-S28-	Gourmet	NON	LED	-20609810.89	4169790.54
10	L15-O38-	Green	Organic	NO	25920656.34	24211837.02
11	L39-S49-	Gourmet	NON	NO	12347577.11	-18494073.89
12	L18-O36-	Gourmet	Organic	NO	12730986.22	6638447.34
13	L79-S44-	Gourmet	NON	NO	-23445342.21	-656281.63
14	L03-O11-	Green	Organic	LED	-14330540.19	9898732.17

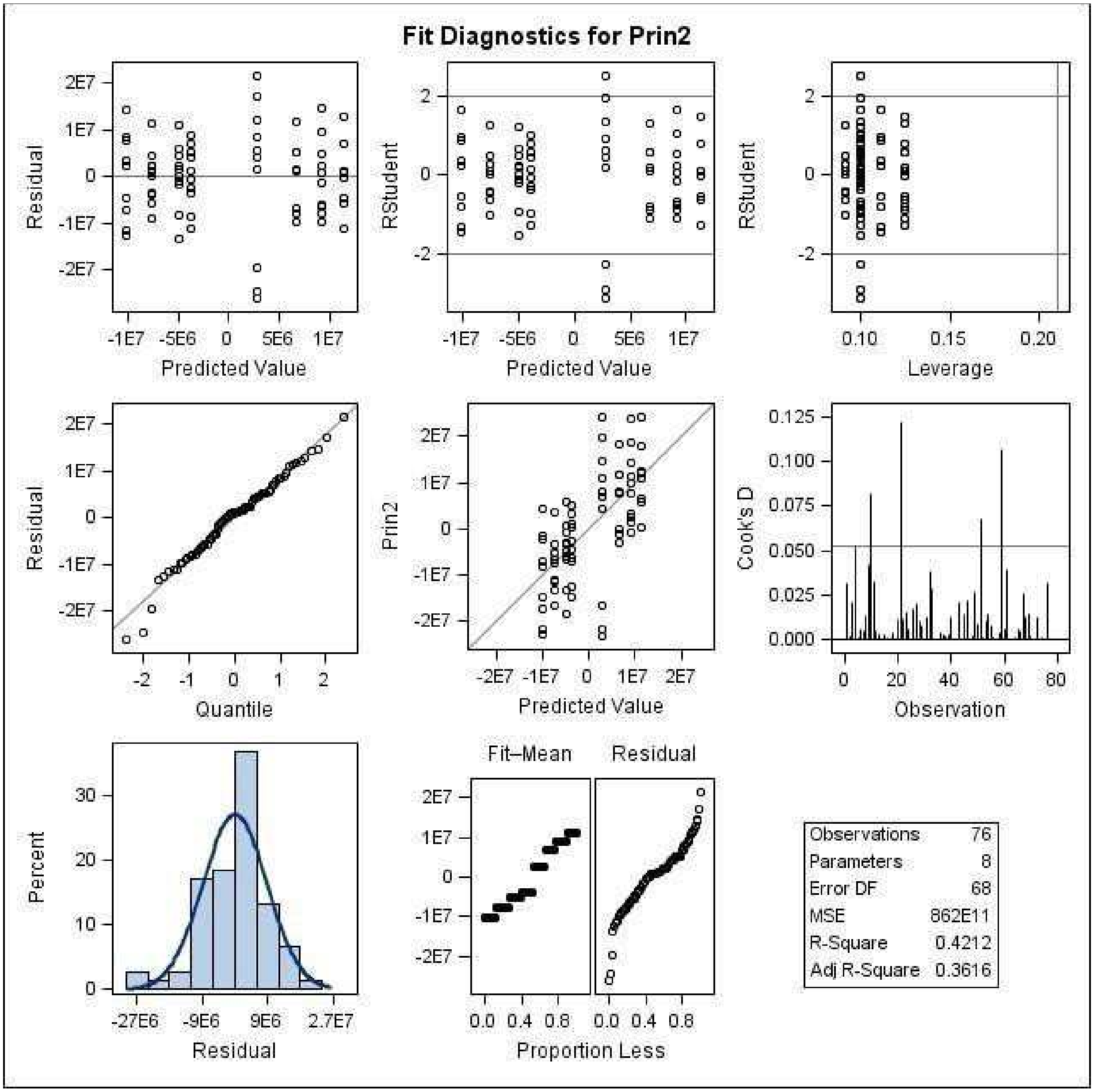
15	L52-O35-	Green	Organic	NO	3538506.07	-6933604.51
16	L69-S23-	Gourmet	NON	LED	5350964.9	-8038455.45
17	L19-O47-	Gourmet	Organic	NO	-5967849.68	12314124.5
18	L68-S8-G	Gourmet	NON	LED	9093604.08	-14879383.5
19	L36-S53-	Gourmet	NON	NO	-28574641.49	-3484095.5
20	L06-O23-	Gourmet	Organic	LED	-28949286.67	-227917.35
21	L12-O35-	Green	Organic	NO	21759392.62	-23359658.21
22	L01-O6-G	Green	Organic	LED	-13844714.16	1291178.91
23	L05-O25-	Green	Organic	LED	-4542162.18	18624201.47
24	L48-O12-	Gourmet	Organic	LED	1337507.26	11698717.4
25	L51-O44-	Green	Organic	NO	-12321550.86	4325484.08
26	L41-O15-	Green	Organic	LED	-3594823.64	-668062.05
27	L23-S26-	Green	NON	LED	-19875078.98	3624613.09
28	L26-S1-G	Gourmet	NON	LED	34467730.29	-17404265.52
29	L04-O27-	Green	Organic	LED	-18294915.5	2670221.4
30	L58-O34-	Gourmet	Organic	NO	16081703.65	12234091.67
31	L22-S11-	Green	NON	LED	27168544.79	-16592167.36
32	L45-O27-	Green	Organic	LED	5034684.6	23786459.19
33	L20-O44-	Gourmet	Organic	NO	12310967.46	197560.73

34	L65-S27-	Green	NON	LED	3249180.57	-6811251.11
35	L43-O9-G	Green	Organic	LED	-19518493.94	7678159.28
36	L35-S35-	Green	NON	NO	-29461398.5	1177830.03
37	L32-S46-	Green	NON	NO	-15635644.39	176244.07
38	L74-S43-	Green	NON	NO	-9181826.29	-7233967.59
39	L63-S11-	Green	NON	LED	14840092.65	-3355485.93
40	L13-O54-	Green	Organic	NO	16456737.13	11091064.75
41	L77-S53-	Gourmet	NON	NO	-13300733.34	-4949687.81
42	L76-S60-	Gourmet	NON	NO	28621032.57	-6105683.69
43	L37-S55-	Gourmet	NON	NO	9462447.47	5819404.67
44	L49-O30-	Gourmet	Organic	LED	-18948045.45	8181362.92
45	L71-S32-	Green	NON	NO	20919150.62	5096386.45
46	L34-S33-	Green	NON	NO	20527243.06	-14944195.59
47	L47-O18-	Gourmet	Organic	LED	-1598630.62	7785692.47
48	L62-S6-G	Green	NON	LED	22591573.95	-11563841.73
49	L27-S22-	Gourmet	NON	LED	-12453847.67	-21691574.35
50	L72-S52-	Green	NON	NO	20998427.99	3137128.43
51	L53-O55-	Green	Organic	NO	7310786.15	-16749749.07
52	L80-S55-	Gourmet	NON	NO	-23341751.95	-2883101.89

53	L17-O59-	Gourmet	Organic	NO	1396531.01	18141589.66
54	L08-O16-	Gourmet	Organic	LED	-19473560.69	-1156341.24
55	L59-O56-	Gourmet	Organic	NO	-5138541.41	5686701.83
56	L25-S9-G	Green	NON	LED	-31142427.81	-5231919.04
57	L56-O37-	Gourmet	Organic	NO	-12948816.98	11007815.43
58	L02-O7-G	Green	Organic	LED	1190907.01	14103596.56
59	L11-O53-	Green	Organic	NO	-35967693.04	-21675027.82
60	L40-S38-	Gourmet	NON	NO	-121476.75	773414.22
61	L16-O49-	Gourmet	Organic	NO	7441103.74	24233283.16
62	L24-S16-	Green	NON	LED	-23124640.58	-7575523.2
63	L75-S48-	Green	NON	NO	12276887.82	-4342804.27
64	L44-O4-G	Green	Organic	LED	-20616692.66	11408558.76
65	L07-O19-	Gourmet	Organic	LED	-5608439.96	11854695.77
66	L64-S16-	Green	NON	LED	-29135934.17	-13164766.83
67	L14-O40-	Green	Organic	NO	21763172.74	14784104.5
68	L70-S7-G	Gourmet	NON	LED	23872728.64	-2375974.66
69	L30-S20-	Gourmet	NON	LED	5535760.26	-1622246.54
70	L61-S22-	Green	NON	LED	-34908740.35	-11122163.16
71	L66-S2-G	Green	NON	LED	-1343496.85	-6568437.38

72	L38-S37-	Gourmet	NON	NO	14479399.16	-13346342.05
73	L78-S39-	Gourmet	NON	NO	20096610.11	-6693581.25
74	L21-S30-	Green	NON	LED	26021708.21	-5172184.46
75	L73-S59-	Green	NON	NO	56163447.38	-2634742.38
76	L46-O26-	Gourmet	Organic	LED	56665038.85	18238731.69




Fit Diagnostics for Prin2
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RStudent
Predicted Value
Leverage
Quantile
Prin2
Cook's D
Observation
Percent
Fit–Mean
Residual
Proportion Less
Observations 76
Parameters 8
Error DF 68
MSE 862E11
R-Square 0.4212
Adj R-Square 0.3616

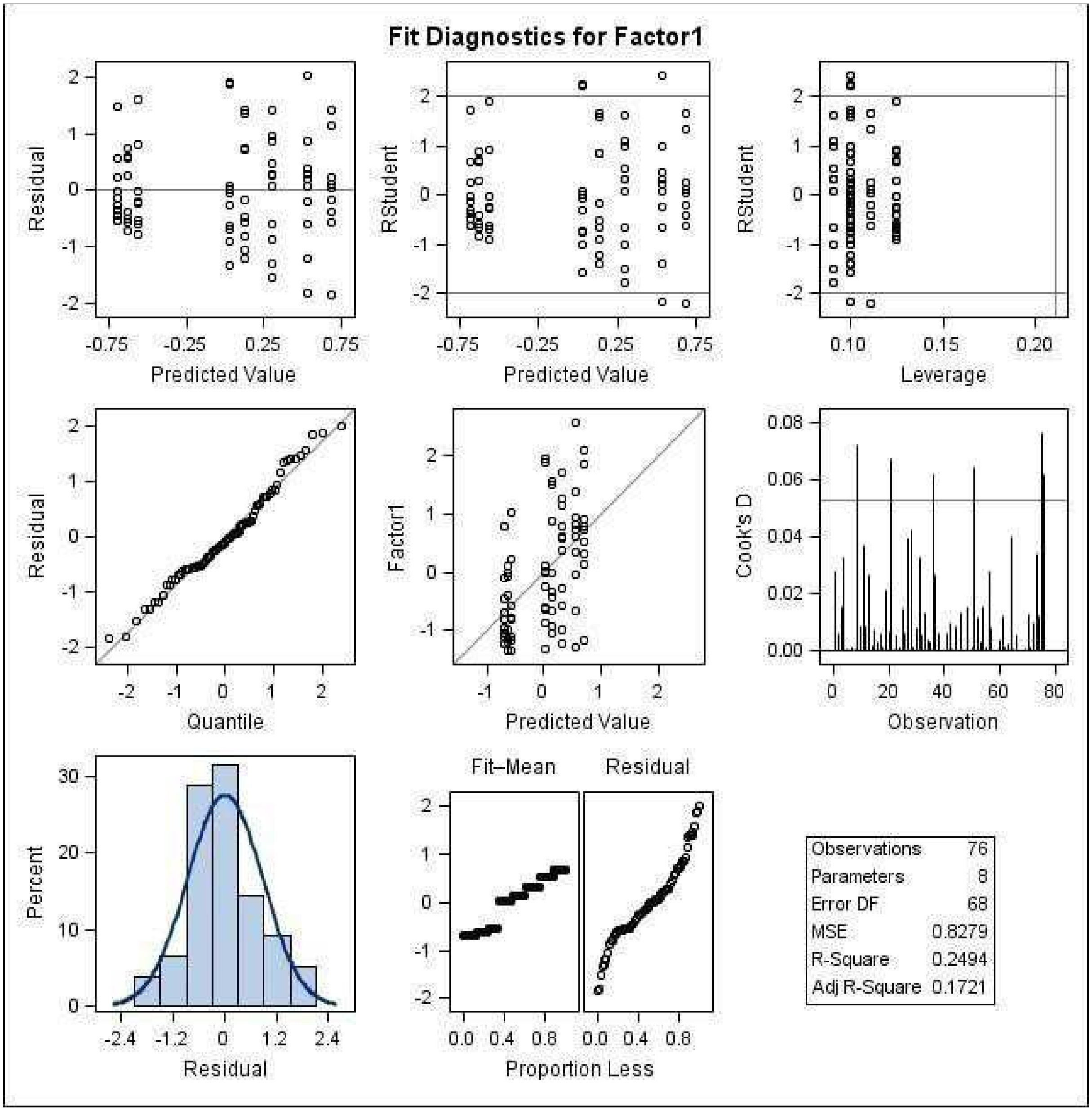
APPENDIX (D) THE FIRST TWO FACTORS' SCORES & DIAGNOSTIC PLOTS FROM ANOVA

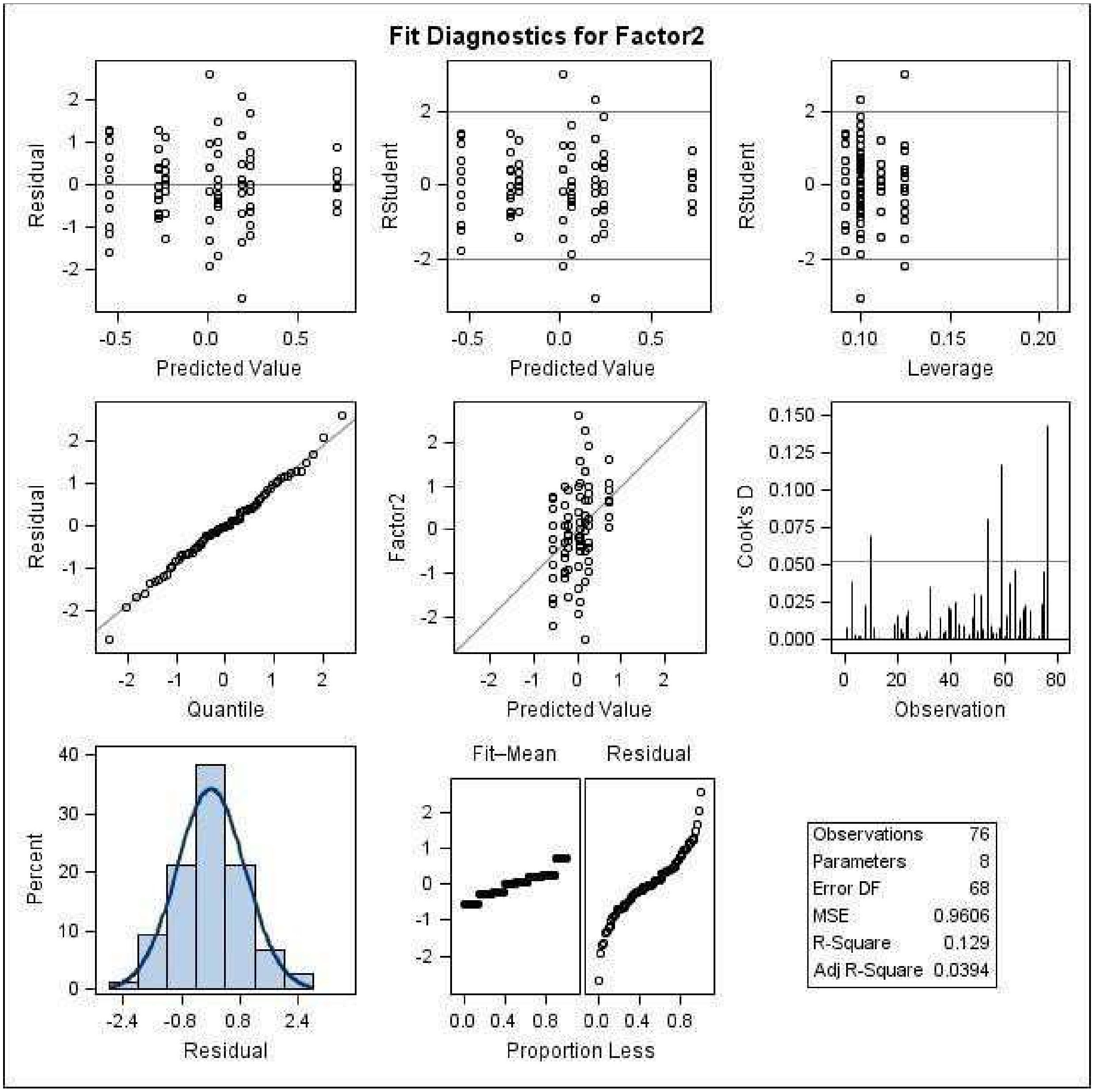
Obs	Analyte	Type	Fertilizer	Light	Factor1	Factor2
~~1~~	~~L28-S13-~~	~~Gourmet~~	~~NON~~	~~LED~~	~~1.83534~~	~~-0.90974~~
2	L67-S4-G	Gourmet	NON	LED	0.13522	-0.09173
3	L09-O21-	Gourmet	Organic	LED	-1.35086	-1.33163
4	L55-O51-	Green	Organic	NO	-1.30326	0.65714
5	L31-S50-	Green	NON	NO	0.72165	0.67776
6	L42-O3-G	Green	Organic	LED	-0.70061	-0.37051
7	L54-O39-	Green	Organic	NO	-0.23594	-0.00778
8	L33-S42-	Green	NON	NO	0.33578	-0.9642
9	L29-S28-	Gourmet	NON	LED	-1.16259	-0.41214
10	L15-O38-	Green	Organic	NO	-0.64784	2.27052
11	L39-S49-	Gourmet	NON	NO	1.54569	-1.00023
12	L18-O36-	Gourmet	Organic	NO	-0.02699	0.66639
13	L79-S44-	Gourmet	NON	NO	-1.06062	-0.625
14	L03-O11-	Green	Organic	LED	-1.03338	0.16474
15	L52-O35-	Green	Organic	NO	-0.60337	0.31811
16	L69-S23-	Gourmet	NON	LED	0.31473	-0.12428
17	L19-O47-	Gourmet	Organic	NO	-1.13789	0.63119
18	L68-S8-G	Gourmet	NON	LED	0.91205	-0.24617
19	L36-S53-	Gourmet	NON	NO	-0.93308	-1.08821

20	L06-O23-	Gourmet	Organic	LED	-1.09638	-0.84773
21	L12-O35-	Green	Organic	NO	1.92423	-0.49475
22	L01-O6-G	Green	Organic	LED	-0.46817	-0.48491
23	L05-O25-	Green	Organic	LED	-1.23052	1.04998
24	L48-O12-	Gourmet	Organic	LED	-0.80302	0.97297
25	L51-O44-	Green	Organic	NO	-0.85949	0.16755
26	L41-O15-	Green	Organic	LED	-0.11512	0.02613
27	L23-S26-	Green	NON	LED	-1.23405	-0.20612
28	L26-S1-G	Gourmet	NON	LED	2.09747	0.27344
29	L04-O27-	Green	Organic	LED	-0.96122	-0.27217
30	L58-O34-	Gourmet	Organic	NO	-0.06715	1.04807
31	L22-S11-	Green	NON	LED	1.7024	0.07508
32	L45-O27-	Green	Organic	LED	-1.22295	1.54471
33	L20-O44-	Gourmet	Organic	NO	0.10828	0.62832
34	L65-S27-	Green	NON	LED	0.7749	-0.45122
35	L43-O9-G	Green	Organic	LED	-1.11419	-0.17402
36	L35-S35-	Green	NON	NO	-1.28505	-0.71449
37	L32-S46-	Green	NON	NO	-0.65961	-0.28406
38	L74-S43-	Green	NON	NO	-0.04935	-0.39287
39	L63-S11-	Green	NON	LED	0.36574	0.69945
40	L13-O54-	Green	Organic	NO	-0.01861	1.33205
41	L77-S53-	Gourmet	NON	NO	-0.43164	-0.51069
42	L76-S60-	Gourmet	NON	NO	0.86419	0.99293
43	L37-S55-	Gourmet	NON	NO	-0.02711	0.55485
44	L49-O30-	Gourmet	Organic	LED	-1.16229	-0.16259
45	L71-S32-	Green	NON	NO	0.61169	1.00063

46	L34-S33-	Green	NON	NO	1.38907	0.0859
47	L47-O18-	Gourmet	Organic	LED	-0.5782	0.41904
48	L62-S6-G	Green	NON	LED	1.2515	0.48153
49	L27-S22-	Gourmet	NON	LED	0.77779	-1.52711
50	L72-S52-	Green	NON	NO	0.81783	0.82221
51	L53-O55-	Green	Organic	NO	1.88408	-1.17437
52	L80-S55-	Gourmet	NON	NO	-0.66104	-0.96034
53	L17-O59-	Gourmet	Organic	NO	-0.99465	0.88677
54	L08-O16-	Gourmet	Organic	LED	0.22784	-1.92859
55	L59-O56-	Gourmet	Organic	NO	-0.38307	0.07169
56	L25-S9-G	Green	NON	LED	-1.00145	-1.10966
57	L56-O37-	Gourmet	Organic	NO	-1.19757	0.2731
58	L02-O7-G	Green	Organic	LED	-0.81069	0.75646
59	L11-O53-	Green	Organic	NO	0.09574	-2.50835
60	L40-S38-	Gourmet	NON	NO	-0.3282	0.10621
61	L16-O49-	Gourmet	Organic	NO	-1.33112	1.59323
62	L24-S16-	Green	NON	LED	0.59509	-2.17113
63	L75-S48-	Green	NON	NO	0.92254	0.23632
64	L44-O4-G	Green	Organic	LED	0.78244	-1.64392
65	L07-O19-	Gourmet	Organic	LED	-0.77098	0.39405
66	L64-S16-	Green	NON	LED	-0.2868	-1.55186
67	L14-O40-	Green	Organic	NO	0.02795	1.33441
68	L70-S7-G	Gourmet	NON	LED	0.72571	0.8977
69	L30-S20-	Gourmet	NON	LED	0.52074	0.08908
70	L61-S22-	Green	NON	LED	-0.57343	-1.70379
71	L66-S2-G	Green	NON	LED	0.57118	-0.81029
72	L38-S37-	Gourmet	NON	NO	0.86007	-0.30911

73	L78-S39-	Gourmet	NON	NO	1.48658	0.09491
74	L21-S30-	Green	NON	LED	1.15368	0.73653
75	L73-S59-	Green	NON	NO	2.55867	1.92506
76	L46-O26-	Gourmet	Organic	LED	1.02165	2.60956

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Cook's D
Observation
Percent
Residual
Fit–Mean
Residual
Proportion Less
Observations 76
Parameters 8
Error DF 68
MSE 0.9606
R-Square 0.129
Adj R-Square 0.0394

APPENDIX (E)

[i] SOP: SOP019, Created: 09/07/2016
Updated: Author: Jacqueline Chaparro
SOP FOR ICP-MS SAMPLE DIGESTION

Materials and Reagents:

1. Use 13x100mm culture tubes to perform your digestions.
a. Location: Shepardson room 302
2. Nitric Acid 70% from BDH CAS # 7697-37-2 VWR # 87003-261
a. Nitric Acid is 70% by weight having a Molarity of 15.9 M (mol/L)
b. Location: Shepardson room 302
3. Hydrogen Peroxide 30% from J.T. Baker CAS # 7722-84-1 Product # 5155-01
a. Location: Shepardson room 302
4. Internal Standard (IS) solution (note 1)
a. 1000 ppm Gallium
b. 1000 ppm Scandium
c. 1000 ppm Bismuth
d. 1000 ppm Yttrium
e. 100 ppm Indium
f. Location: Shepardson room 302
5. Pure water 18MΩ or better. The Researcher have tested the PMF distilled, Shepardson distilled, and soils lab distilled water and all have very low background.
6. Automatic or normal pipette
7. Pipette tips
a. Plastic is fine. You must replace the tip after every sample.
8. 15mL centrifuge tubes made of polypropylene for dilutions.
a. Location: Shepardson room 302

Protocol: For plant material (method optimized for wheat grain, barley, potato, onion)
Please, read all notes before beginning protocol!

1. **______**Weigh out **100mg (0.1g) to 150mg** (0.150g) dry sample into digestion tube (13x100mm culture tubes).
2. **______**Record the exact weight of each sample
3. **______**Label at least three tubes as **BLANK.** These tubes will act as your blank solutions. (note 2)
4. **______**Add 66.7uL of 10ppm IS mix to each sample and blank from the IS premade mix (see above) with Sc, Y, Ga, Bi, and In. This amount will produce a **FINAL** concentration of 20ppb for each internal standard.
5. **______**Add 1.5mL nitric acid (trace metal grade 70%) to each tube and cover with plastic wrap and let sit overnight.

6. ______Place tubes (samples and blanks) on sand bath and heat at approximately 120°C for 2.5 hours. Samples will create a red/orange smoke (note 3)
7. ______Remove tubes from sand bath let cool for approximately five minutes.
8. ______Add 750µL 30% hydrogen peroxide.
9. ______Place tubes back in sand bath, and heat for 1hr at 120°C. (note 4)
10. ______Remove samples from block, cover and let cool for approximately five minutes.
11. ______Transfer solutions into 15mL falcon tubes (can just pour them in directly).
12. ______Raise volume to 10 mL using 18MΩ water (note 5) at this point you have a solution at 10% HNO_3 and an IS concentration of 0.0667 ppm.
13. ______Take 4.5mL of above solution and add to a new 15mL falcon tube.
14. _ Dilute above 4.5mL with 18MΩ water to a final volume of 15mL (using the 5mL pipette usually add 5mL two times) IS final concentration of 20 ppb and 3% HNO_3. (note 6)
15. ______Prepare QC samples (note 7). Take 1mL of sample from each unknown **not** including blanks and adding it to 50mL falcon tubes (note 8, note 9).
16. ______Make sure to prepare your standards on the day of analysis at 3% HNO_3 final concentration and 20ppb of IS. (see SOP0XX .docx)

Notes:

1. Internal Standard mix (IS) that consists of one or all of the following elements: make your own from individual stocks 100ppm In, 1000ppm Ga, Bi, Sc, and Y.
a. **Only** make a stock of internal standard solution of the internal standard elements you will be monitoring.
b. To make your own from In, Ga, Bi, Sc, and Y. Mix 150µL each of Y, Ga, Sc, and Bi (found at a 1000ppm stock concentration) and 1.5mL of In (found at a 100ppm concentration) and raise volume with 18MΩ water to 15mL (make sure 15mL is enough for all your samples and standards. This makes a 10ppm stock solution which you will be adding to each sample, blank, and standard.
2. You **have to** run one blank before each standard curve. (15mL of blank is usually enough for two independent runs and throughout the runs to monitor and correct for carryover.
3. After 2.5 hours, smoke in tubes should be pretty clear (not orange). If it is still very orange, let the samples cook for longer. Once clear, move on to step 5. If you go on for longer just record the amount of time it took and make sure all samples are exposed to the same amount of time.
4. Solution should be clear or light yellow in color everything should be digested.
5. Check but usually you obtain 2mL of sample and have to add 8mL of water, using the 5mL pipette add 4mL two times)
6. Mainly be consistent and careful to pipette the same amount every time.
7. For **QC** samples you have the following options:
a. Make a 20ppb final concentration of all the elements including internal standards you will be analyzing
b. Combine ~1mL of each of your unknown samples and mix together into an appropriate container 50mL falcon tube (This is the better method use this method unless otherwise noted)

8. If 50mL falcon tube is not big enough to hold the entire QC sample use multiple 50mL falcon tubes and mix them amongst each other to ensure homogeneity.
9. Determine the number of QC samples you will need based on how many total samples you have. Ensure that you have enough QC sample to run once every 10 samples. If running the essential biological method you will need approximately 7mL per QC sample. QC samples are the first and last samples run. For QC we like to run at least one QC sample after every 10 samples have been run.

<u>Calculations:</u>

<u>Dilution factor correction</u>

Dilution Factor: (final volume)/(initial volume)
In above sample digestion dilution factor is as follows:
DF=(10mL)/(100mg)*(15mL/4.5mL)=0.3333mL/mg

To correct for dilution factor the following calculations are performed:
If after calculating the concentration based on the standard curve your units are ppb and you want your answer in ppb do the following:
Example1: analysis reveals 10.5 ppb of Cu without dilution factor correction
10.5ppb * (10mL/(100mg/1000))*(15mL/4.5mL)=3500ppb
10.5ppb * (10mL/100mg)*(15mL/4.5mL)=3.5ppm
Example 2: Internal standard of In Final concentration is 20ppb in diluted solution what was the stock
20ppb* (10mL/(66.7mg/1000))*(15mL/4.5mL)=9995ppb
20ppb * (10mL/66.7mg)*(15mL/4.5mL)=9.995ppm NOTE: Stock was 10ppm solution (see above)