The use of rock dust as a natural media amendment for the production of horticultural crops in controlled environments

By

Abraham Armah

A thesis submitted to the School of Graduate Studies

In partial fulfillment of the requirements for the degree of

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Boreal Ecosystems and Agricultural Sciences

School of Science and the Environment

Grenfell Campus, Memorial University of Newfoundland

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St. John’s Newfoundland and Labrador

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Approved By:

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Supervisor: Dr. Raymond Thomas Co-Supervisor: Dr. Lord Abbey

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General Summary

Rock dust (RD) is a by-product of the mining industry generated after extracting precious minerals like gold. However, RD has low commercial use and is typically stocked-piled at mine sites for disposal. Potentially, RD contains macro- and micronutrients required for crop growth. Thus, RD could be a valuable substrate for amending crop growth media, thereby improving productivity and enhancing economic and environmental benefits. This study aimed to evaluate the potential of RD as a natural soil amendment for improving media quality, agronomic performance, and nutritional quality of vegetable crops produced under controlled environmental conditions. The controlled environmental pot experiments were carried out in a walk-in growth chamber located at Grenfell Campus, Memorial University of Newfoundland. Experimental treatments contained 10 different media formulations amended with RD and were denoted as follows: 1) 100%Rock dust (RD), 2) 50%Rock dust+50% Topsoil(RDT), 3) 50%Rock dust+ 25%Biochar + 25% Promix (RBP) 4) 100%Topsoil(TS), 5) 25%Rock dust + 75% Topsoil(RT), 6) Huplaso (H) (negative control), 7) 50%Rock dust+ 25% compost +25% Promix (RCP)8)50%RD+ 50%Promix (RP), 9) Promix (Control), 10) 50%Rock dust + 50%Biochar(RB). The experiment was a completely randomized design (CRD) with four replications and three crops (amaranth, kale, and lettuce) per treatment. Agronomic performance evaluated included chlorophyll content, root shoot ratio, biomass yield etc.), growth media quality included active microbial community structure, mineral composition, porosity, bulk density, pH, and field capacity.), and crop nutritional quality was represented by phenolic content, antioxidant activity, mineral nutrients, and fatty acid composition. We observed six different microbial communities, namely G+ & G- & Fungi (F), protozoa, and eukaryote, and a strong relationship between the physicochemical properties of the media and the active microbial composition. These include high correlations between the bulk density and the microbial composition. The best agronomic performance was observed in RCP, RBP, RB, and RP. This includes increased total biomass, chlorophyll content, number of leaves, and fresh weight. The RD-based media amendments, RCP, RBP, RB, and RP successfully modulated horticulture crop nutritional composition (fatty acids, protein, minerals, and antioxidants). We next tried to assess the relationship between media quality and how this influences the crop agronomic performance and nutritional quality. We observed substantial positive correlations(r=0.88) of BD (bulk density) with RSR (root-shoot ratio), a negative correlation(r=-0.81) of BD with G+&G-&F, a positive correlation(r=0.74) of porosity with FW (Fresh weight), and strong positive correlation of TBM (Total biomass) with MUFA (Monosaturated fatty acid) and a strong positive correlation of BD with total macro minerals in the plant tissue. Mechanistically, the strong associations observed between the media quality and crop agronomic performance as well as nutritional quality suggest that RD-based media have superior bulk density, porosity, nutrient composition, and microbial composition. This resulted in crops having an enhanced capacity to absorb nutrients from the media mineralized by G+&G-&F and translocate this into superior biomass and bioaccumulation of the following nutrients: protein, MUFA, total antioxidant, and total macronutrients. Our results demonstrate that using RD as natural media amendment could provide a cost-effective way of providing a high-quality growth media for producing vegetables with superior yield or biomass and improved nutritional quality under controlled environmental conditions. RD-based natural media amendments could provide a sustainable alternative for the by-product disposal.

Keywords: mine by-product, rock dust, soil amendment, active microbial composition, soil health, crop nutritional quality, crop growth performance

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List of Abbreviations

%= Percentage

°C= Degree Celsius

1P=Protozoa

ABTS=[2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)]

Al = aluminum

ANOVA= Analysis of variance

As= Arsenic

B = Boron

Ca = Calcium

Cd = Cadmium

CEC=Cation exchange capacity

cm = Centimeter

Co = Cobalt

Cr = Chromium

CT=Chlorophyll content

Cu = Copper

DW=Dry weight

EC=Electric conductivity

Fe = Iron

FRAP = Ferric reducing ability of plasma

FW-=Fresh weight

g = Gram

G-= Gram-negative bacteria

G+ = Gram-positive bacteria

G+/G- = Gram-positive bacteria / Gram-negative bacteria ratio

GC=-FID – Gas chromatography – Flame ionization detection

GovNL–Government of Newfoundland and Labrador

H = Huplaso

HAA = Hydrophilic antioxidant content

HNO_3 = Nitric Acid

ICP-OES = Inductively Coupled Plasma Optical Emission Spectroscopy

K =Potassium

kg = kilogram

LAA =Lipophilic antioxidant content

LSD=Fisher's least significant difference

LT=Leaf temperature

Mg = Magnesium

mg=milligram

Mn = Manganese

Mo = Molybdenum

MS=Mass spectrometer

Na = Sodium

Ni = Nickel

NL=Number of leaves

P = Promix

PCA=Principal Component Analysis

pH = Potential of hydrogen (Hydrogen ion concentration)

PLH=Plant height

P_L=Phosphorus in the leaf

P_S = Phosphorus in the soil

PUFA=Poly unsaturated fatty acid

Q1 = First quadrant

Q2 = Second quadrant

Q3 = Third quadrant

Q4 = Forth quadrant

QE = Quercetine equivalent

RB =50%Rock dust + 50%Biochar

RBP = 50%Rock dust +25%Biochar +25% Promix

RCP=50%Rock dust +%25Compost +25%Promix

RD =100% rock dust

RDT=50%Rock dust +50% Topsoil

RP =50% Rock dust +50%Promix

RSR=Root-shoot ratio

RT=25%Rock dust +75%Topsoil

S = Sulphur

Se = Selenium

SE = Standard error

SFA=Saturated fatty acids

SPAD = Soil plant analysis development

TAA = Total antioxidants

TE = Trolox equivalents

Ti = Titanium

TPC = Total phenolic contents

TS = 100% Topsoil

Zn = Zinc

μg/mL = Microgram per milliliter

μL = Microliter

μmol L^{-1} = Micromoles per liter

Structure of the Thesis

This thesis follows a manuscript format and consists of four chapters.

Chapter 1: An introductory chapter to this research, including objectives and structure of the thesis.

Chapter 2: A standalone manuscript highlighting the physiochemical properties of rock dust as natural media amendment. The paper reports on the formulation of rock dust media amendment and analysis of the nutritional value of these media formulated.

Chapter 3: A second standalone manuscript reveals rock dust-based amendment's performance in producing kale, amaranth, and lettuce in a controlled environment. The paper also reports on the biochemical analysis of the produce, and the correlations of the media quality, agronomic performance, and mineral composition. Finally, the study demonstrated a possible mechanism of how RD-amendments media quality influenced crop growth, nutritional composition.

Chapter 4: Includes the general summary of the entire thesis and future recommendations to further progress the research.

CHAPTER 1

1 Introduction

1.1 Food production, mining industry, and environmental impact

The UN predicted the world population to increase by 2.25 billion and reach 9.5 billion by 2050 (Alexandratos and Bruinsma, 2012). The current projection and increase in world population associated with demand for food have resulted in the use of various technologies to improve food production. The excessive use of synthetic fertilizer adversely affects the environment and ecological functions such as soil degradation and loss of soil fertility. As a result, crop productivity is reduced, leading to increased food and nutrition insecurity. (Ahmed *et al.*, 2017).

Furthermore, the mining industry contributes to adverse environmental impacts associated with their extraction and processing activities, urban expansion, land displacement, and disposal of a large amount of waste materials in the environment (Sonter *et al.*, 2017b). The mining industry activities contribute to the loss of biodiversity, access to arable land for agriculture production, and potential human health issues (Sonter *et al.*, 2018). This mining activity is particularly more evident in rural communities that are the traditional locals for these mining operations.

Anaconda Mining Inc is a precious metal (gold) mining operation located on the Baie Verte peninsula (49°57′42″ N, 56°07′23″ W) in rural Newfoundland and Labrador (NL). This operation produces approximately 2 million tons of RD annually as a waste by-product from their (gold) mining operation, which has potential for commercial use. Currently, the RD by-product is stockpiled at the mining site with no existing commercial use for it. Preliminary investigations determined the chemical and mineral composition of this RD by-product to be within acceptable limits for vegetable crop cultivation with unremarkable levels of toxic heavy metals. The heavy metal and cyanide levels observed are within the safe limits suggested by the Canadian Council of

Ministers of the Environment (CCME) for agricultural media. (Arnott *et al.*, 2021). This evidence suggested that the RD by-product from Anaconda mining operations may have properties suitable as a media amendment for vegetable crop production. Considering only 10 percent of the food (including vegetables) consumed in the province is produced here, leading to ongoing challenges with nutrient and food security, particularly in remote rural communities of Newfoundland and Labrador.

Furthermore, year-round local vegetable production is a major challenge due to poor soil fertility (i.e., coarse-sandy texture and low pH (4.0 - 4.5) (Amundson *et al.*, 2015), short growing season, and extreme weather conditions (Sauer *et al.*, 2007). In finding a solution to the above problems, it has become critical to adopt sustainable and sound environmentally friendly practices to enhance the province's food production. Hence, the potential exists to explore the suitability of RD from Anaconda mining as a suitable media amendment to address the poor soil fertility and low vegetable production challenges currently experienced in NL. The RD has been found to improve soil pH, and contains available nutrients for plant growth(Arnott *et al.*, 2021).

Recently the Government of Newfoundland (GovNL) has announced "The Way Forward on Agriculture" work plan. The main agriculture target of the GovNL is to reach 20% food production by 2023 to improve food security in the province. Innovative and sustainable cropping systems involving integrated organic and inorganic inputs as soil amendments could be valuable to improving agricultural productivity on marginal soils or under controlled environmental conditions (Selim, 2020). Utilizing industrial by-products, including RD, as a growing media amendment will improve agricultural soil nutrient status and health. Providing potting media for crop production under controlled environmental conditions has gained a lot of recent interest in the scientific community. Application of RD to croplands could affect the physicochemical and biological properties of the soils, but the effects may not be apparent over short periods. Dall'Agnese et al. (2014) reported that the slow release of available nutrients from RD can increase crop yields in subsequent years. RD has been reported to have the potential to supply crops with nutrients for their growth when incorporated into the soil (Ramos *et al.*, 2017). RD maintains a nutrient reserve in the soil in the form of a crystalline structure after the crops have taken up the necessary nutrients for growth (Gindri Ramos *et al.*, 2021). This could serve as an economic benefit for the agricultural production industry.

1.2 Soil re-mineralizer

The global demineralization of agricultural soils, mainly due to intensive industrial development, is of significant threat to food security (Belliard *et al.*, 2014). The rate of decline in soil fertility exceeds its natural mechanism of replenishing these lost nutrients (Amundson *et al.*, 2015). Crops require 14 essential elements (calcium, nitrogen, magnesium, phosphorus, potassium, copper, zinc, Iron, boron, molybdenum, sulphur, nickel, manganese, and chlorine) for optimum growth (Oliver and Gregory, 2015). Soil restoration and sustainable nutrient management is a robust approach for sustainable crop production. Previous studies have shown that combining agronomic practices like crop rotation, crop residues, and organic waste incorporation can improve soil fertility (Ray *et al.*, 2020; El Janati *et al.*, 2021). However, these practices may not be sufficient to enhance soil mineral restoration. They will require an integrated approach of incorporating organic and inorganic fertilizer to improve soil fertility and positively impact food production (Oliver and Gregory, 2015).

Stone meal Technology has been practiced as one of the sustainable and natural practices of re-mineralizing the soil. It is a process of using rock dust as a soil re-mineralizer which is viewed to be more sustainable and effective based on the positive results from many studies reported in the scientific literature. RD was used as a natural fertilizer and applied alone or mixed with NPK in an experiment conducted over three years (Leonardos and Theodoro, 1999). The results showed that only RD application and the mixture treatment produced significantly higher plant yield than the crops fertilized with only NPK.

Furthermore, RD has been reported to significantly improve the growth of beans, arugula, and quinoa (Theodoro *et al.*, 2021). Five different formulations of RD amendment, including RD only, NPK, organic fertilizer formulation, and a mixture of RD were used. The results showed a significant increase in crop growth in RD treatments compared to the control and the other non-rock treatments for all the crops evaluated except for beans and arugula. Other studies conducted in Australia, India (Basak *et al.*, 2020), Brazil, and the United Kingdom (Manning, 2018) have reported RD as an effective soil remineralizer. These studies contrast to other reports suggesting that rock dust is an ineffective soil remineralizer (Ramezanian *et al.*, 2013). For example, their work demonstrated that RD application had no significant effect on wheat growth, nutritional composition, and soil active microbial community composition.

Campbell (2009) conducted experiments using RD and green manure compost as soil amendments in field and greenhouse for three years. The results showed RD application had no significant difference in yield and plant nutrient levels in both fields and the greenhouse trials compared to green compost. Nonetheless, RD has a chemical and mineral composition that can improve the remineralization process when added to poor soil, and the extent can vary with crop cultivated.

1.3 Effect of rock dust on soil pH

Soil pH is the measure of the acidity or alkalinity of the soil, which can be related to many different soil properties.[Soil pH is considered a master variable that impacts soil organic matter decomposition, physical properties, and nutrient cycling, influencing plant development and biomass yield (Neina, 2019). Ramos *et al.* (2020b) had shown a significant increase in pH when RD was mixed with poor soil (low pH) after 140 days of cultivating maize and black oat. Additionally, Dumitru *et al.* (1999) revealed the gradual increase in soil pH by RD over time. RD's increase in soil pH can be attributed to the dissolution of various minerals by the activities of soil microorganisms that exist in the root rhizosphere (Ribeiro *et al.*, 2020).

1.4 Effect of rock dust on Microorganisms

Soil microorganisms also require macronutrients and micronutrients as substrates and are essential in the soil-water-plant continuum to maintain good soil health by recycling nutrients. Many authors have reported the potential value of RD amended media to enhance the soil microbial activity, improve plant growth and soil health. For example, RD has been shown to increase microbial activity in different media (Li *et al.*, 2020), environments (Certini *et al.*, 2004), and field soil (Faraone and Hillier, 2020). Furthermore, RD amended media improved vegetable crop growth and yield under controlled environmental conditions (Abbey *et al.*, 2019). It is known that RD may vary in their physical, chemical, and biological properties due to differences in their parent rock material, soil formation process, vegetation, and climatic factors. Anaconda Mining Inc. produced approximately 2000,000 tons of RD annually from its operation in Baie Verte, NL. This RD from a volcanic source may have properties suitable as a natural media amendment for crop production.

1.5 Rock dust as a potential natural media amendment

It has been reported that RD can contain some level of macro-nutrients (phosphorus (P), sulphur (S), calcium (Ca), magnesium (Mg), and potassium(K)) and a range of micro-nutrients boron (B), chlorine (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), zinc (Zn)) required for optimal crop growth. Furthermore, RD can also improve soil's physical, chemical, and biological properties (Van Straaten, 2006). In addition, it can also improve the water holding capacity of the soil (sandy and clay soil) (Sun, 2018) and maintain soil aeration, which is vital in improving soil health and crop productivity. The incorporation of rock dust and compost into poor acidic soil has been found to significantly enhance the growth of crops such as apple fruit production (Li *et al.*, 2020)..

1.6 Project goal and objectives

The overall goal of this study is to evaluate the potential of RD as a local natural media amendment or alternative nutrient source to boost vegetable production and quality in a controlled environmental condition in Newfoundland and Labrador province (NL). The use of innovative and sustainable cropping systems involving organic and inorganic fertilizer could be a practical approach to improve agricultural productivity in marginal soils or controlled environments (Fang *et al.*, 2021). As such, utilizing industrial by-products, including RD as amendments to improve agricultural lands' nutrient status and health and provide potting media for crop production under controlled environmental conditions, have gained a lot of recent interest in the scientific community and worldwide(Beerling *et al.*, 2018). The slow release of available nutrients from rock dust can increase crop yields in subsequent years. However, there may be limitations to the short-term evaluation of the actual agronomic value of these materials as amendments (Diacono and Montemurro, 2011)

Most of the agricultural lands in Newfoundland have podzolic soils. It has contributed to insufficient food production leading to, for instance, high importation of vegetables from the mainland and other countries into the province to meet food demand. In other to find solution to the food security issue, which is on the increase in the province, wee need to improve our knowledge and understanding of other alternative and environmentally friendly approaches to sustainably increase nutrient-enriched food production in the province. One practical approach is utilizing natural soil amendments in controlled environment crop production. This study aimed to evaluate the potential of RD as a soil amendment to produce horticultural crops under controlled environmental conditions. It is hypothesized that; RD from Anaconda mining operations would be a valuable natural media amendment for crop production.

The objectives of the study were:

1) To evaluate the RD's nutrient composition and physicochemical properties to determine benchmarks before media formulation.
2) To evaluate RD as a natural media amendment to produce Kale, Lettuce, Amaranth in a controlled environmental condition.
3) To evaluate the active microbial composition of RD formulated media
3) Determine the nutritional quality of crops produced with RD-based amendments.

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CHAPTER 2

Evaluation of the physicochemical and microbial properties of rock dust amended potting media formulations

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Abstract

Rock dust (RD) is a major by-product of the precious metal mining industry which contains macro- and micronutrients. Anaconda produces close to 2,000,000 tons of RD as a by-product, which is a stockpile with no commercial use. This poses environmental issues and mining operational costs. This study is aimed to evaluate the physicochemical properties of RD, active microbial community composition, and potential applications in the formulation of RD-based media amendments. Experimental treatments were 1) 100%Rock dust (RD), 2) 50%Rock dust+50% Topsoil (RDT), 3) 50%Rock dust+ 25%Biochar + 25% Promix (RBP) 4) 100%Topsoil (TS),5) 25%Rock dust + 75% Topsoil (RT), 6) Huplaso (negative control), 7) 50%Rock dust+ 25% compost +25% Promix (RCP)8)50%RD+ 50%Promix (RP), 9) Promix (Control), 10) 50%Rock dust + 50%Biochar(RB). We evaluated their physicochemical properties, active microbial community composition, and suitability for crop growth. The RD particle size particle ranged from 0.1-2 mm. The bulk density of RD was 1.5 g $cm^{-3,}$ and the values ranged from 0.5 to 1.1 g cm^{-3} in the RD-based media amendments with an increase in porosity. Assessment of the active microbial compositions reveal six classes of microbes (Protozoa. Eukaryotes, Gram-positive, Gram-negative, and fungi) were identified with a significant increase in G- & G+ & Fungi (F) in RD-based amendment RBP (28.38±4.88%) compared to the control P (9.49±0.06%). We observed a strong negative correlation (r= -0.98) of G+ & G- & F with pH, a strong positive correlation (0.85) of Ca with eukaryotes, and a strong positive correlation (r=0.95) of CEC (cation exchange capacity) with G+. This research suggests that effectively blending RD with organic amendments could be a potential source of macro-and microminerals for agriculture soil and plants growth**.** These findings could be vital for media development and the production of vegetable crops.

***Keywords*:** Active soil microbial composition, natural media physicochemical composition, precious metal mine by-products, volcanic based RD, bulk density, control environment cropping systems.

2.1 Introduction

The mining industry produces about 7-17 billion tons of RD (Basak *et al.*, 2020) as by-products associated with detrimental effects if not handled properly. For instance, RD from the precious mining industry is considered a waste by-product and is usually dumped on landfill sites. Such disposals of RD are of significant environmental and financial concerns and can impact aquatic ecosystems, landscape, and soil health (Sonter *et al.*, 2017a). Hence, the pressing questions about safe handling or sustainable management of a significant volume of RD are essential to reduce the environmental and financial burden on the industry. Previous findings have revealed that RD contains several mineral nutrients required for plant growth, and there is a growing interest in assessing the potential of RD as growth media.

RD is a pulverized particulate of volcanic rock obtained as a by-product from precious metal mining. Ramos et al. (2019) reported that RD contains several minerals in the required quantities to support plant growth. The information in the literature is contradictory about the potential of RD as a suitable nutrient source or growth media to augment crop growth, development, and crop yield. For instance, few studies revealed little or no RD effect on crop growth (Bakken *et al.*, 2000; Bolland and Baker, 2000; Campbell, 2009; Ramezanian *et al.*, 2013), while other studies showed RD successfully improved the yield of maize(Ramos *et al.*, 2020b; Basak *et al.*, 2021). The successful improvements of plant growth by RD amendments are evidence of a high concentration of magnesium (Mg) and calcium (Ca), increased level of Ca, K, and phosphorus (P) in soil, as well as low level of aluminum (Al). Several studies on volcanic RD have shown that RD contains about

17 essential minerals, including the macro and micronutrients except for nitrogen (Swoboda, 2016; Gindri Ramos *et al.*, 2019; Ramos *et al.*, 2020a; Plata *et al.*, 2021).

The use of RD amendments has been demonstrated to raise soil pH from 5.13 to 6.81, as well as the CEC from (149.44 mmol/kg to 153.43 mmol/kg) resulted in an improvement in plant growth (Li and Dong, 2013). Newfoundland and Labrador (NL) face challenges of local food production due to acidic and shallow soils and extreme weather conditions. Therefore, there is a need to develop and assess RD growth media formulations and soil amendment to improve physicochemical properties to enhance food production while minimizing adverse effects on soil ecosystems. Hence, creating an improved growth media that can be used indoors or in a greenhouse to produce high-value crops which would provide a better handling of RD, thereby increasing crop production to meet the growing food needs.

In northern or boreal regions, food insecurity challenges have led to an interest in developing alternative production systems to provide sustainable food supply to support the growing population. Precious metal mining is a major resource extraction industry located in rural northern communities where there is a significant accumulation of RD as a by-product of the mining operation. The evaluation of RD's chemical, physical and biological properties are of interest as a suitable growth media amendment to produce crops under controlled environmental conditions in boreal or northern climates. Our industry partner in this study currently has over 2 M tons of RD annually as an industrial mining by-product. It is hypothesized that RD is a suitable natural media amendment that will improve the chemical, physical, and biological properties that will support plant growth under controlled environmental conditions. We, therefore, aim to evaluate: 1) the physical and chemical properties of RD and RD-based amendments 2) the microbial community composition and abundance of RD and its influence on formulated media quality and suitability for plant growth.

2.2 MATERIALS AND METHODS

2.2.1 Experimental site, collection of samples, and preparation

The experiment was conducted in a walk-in growth chamber located at the Boreal Ecosystem Research Facility, Memorial University of Newfoundland. RD was obtained from deposits at Anaconda Mining, Baie Verte, Newfoundland (Latitude 49°57′42″ N, Longitude 56°07′23″ W). The 2-year study was carried out between May 2019 and May 2021. First, RD was collected in transects from different areas across the collection site and chemically analyzed to determine element of environmental concern (e.g., mercury, lead cyanide etc.) After the chemical analysis and the safe levels were ascertained from the analysis, RD was used to formulate 10 media amendments as follows: 1) 100%Rock dust (RD), 2) 50%Rock dust+50% Topsoil(RDT), 3) 50%Rock dust+ 25%Biochar + 25% Promix (RBP) 4) 100%Topsoil(TS),5) 25%Rock dust + 75% Topsoil(RT), 6) Huplaso (negative control), 7) 50%Rock dust+ 25% compost +25% Promix (RCP) 8)50%RD+ 50%Promix (RP), 9) Promix (P)(Control), 10) 50%Rock dust + 50%Biochar (RB).

2.2.2 Media preparation

The soil and other media were air-dried for 48 h, volumetric flask (1000 mL) was used to measure and standardize the quantity of the media components on a volume basis. A volume of 1000 mL was considered 100% composition, and 500 mL was considered 50% of the total composition, and ten formulations mentioned above were prepared with the respective mix ratios. Each of the mixtures was thoroughly mixed by hand to ensure uniform media mixing. The combinations were then filled into 12.95 cm x 13 cm diameter planting pots.

2.2.3 Particle size analysis

The RD material was oven-dried at 105 °C for 48 h (Shel lab, Sheldon Manufacturing. Inc., 300 N.26TH Cornelius, USA). Particle size distribution analysis was conducted using the wet sieve method by Blaud *et al.* (2012) with slight modification (**Fig.2** 1). A sieve set of ASTM standard (size ranges from largest of 500 μm to the smallest sieve size of 45 μm) was used. The coarse screen at the top has a 425-micron opening. Its opening is 180,000 square microns in size. The openings at the down parts are half the size of the one above. As a result, the opening area for the second screen down is 90,000 microns. The large particle size >2mm diameter was removed before weighing. Aliquots (100 mL) of distilled water were used to wash the soil, and water was collected at the bottom of the sieve set.

The sieved soil particle collected on each sieve was brushed into an aluminum pan and oven-dried at 105 °C and allowed to cool. Then, samples were weighed and calculated the percentage weight of each fraction of particles. A cumulative particle-size distribution curve was developed using the average of three replicates.

2.2.4 Bulk density, field capacity, and porosity

Basic physical soil properties (bulk density, porosity, field capacity) of RD were determined following the method of Saha *et al.* (2020). The mass of three (replicates) empty metal rings (M_1) was taken, and the metal rings were filled as uniformly as possible with the media by tapping three times on the table. The weight of the metal ring with soil, including filter paper and the rubber band, was recorded as (M_2). The samples were saturated for two days by gradually absorbing water from the bottom of the sample. Samples were kept in an aluminum container, and water was added to the container initially to cover 1/3 of the sample height. Then, after around 8 h, water was again added to the container to cover 2/3 of the sample, and finally, the water level was maintained just below the brim of the metal ring. When water film was observed on the sample's surface, it was said to be saturated. The saturated sample was weighed (M_3) and then placed on an empty metal ring to freely drain for 1-3 days before recording the final weight (M_4). The surface was covered with plastic wrap to avoid surface evaporation. Samples were oven-dried at 105 °C to obtain the dry mass (M_5).

Bulk density was calculated by dividing the mass of the dry sample (M_5) by the volume of the can containing the sample. Porosity was calculated by subtracting the mass of the dry sample from the weight of the saturated sample (M_3-M_5) and dividing it by the sample volume. Field capacity was calculated by subtracting the mass of the dry sample from the weight of the drained sample (M_4-M_5) and divided by the sample volume. When calculating the porosity and field capacity, the density of the water was assumed as= 1 g/cm^3.

2.2.5 Media formulation with RD amendment

There were ten different treatments, out of which seven were amended with RD. Details about the treatments are given in Table 2.1.

Table 2.1: Treatment of RD and RD-based media formulations with code and the combination level.

Treatment code	Treatment	Combination level
P	Promix^{TM}	100%
RBP	RD+ biochar + Promix^{TM}	50%+25%+25%
H	Huplaso	100%
RT	RD + topsoil	25% +75%
RP	RD+ Promix	50% + 50%
RCP	RD+ compost + Promix^{TM}	50%+25%+25%
RD	RD	100%
RDT	RD+ topsoil	50% + 50%
RB	RD+ biochar	50% + 50%
TS	Topsoil	100%

The media were first air-dried and measured with a 1000ml laboratory beaker into a container and thoroughly mixed to have a uniform mixture. The mixture was then transferred into 12” plastic planting pots for transplanting.

2.2.6 Rock dust pH analysis

Three replications of samples were taken from each of the formulated media amendments. In determining soil pH, the 1:2 method was adopted, as reported by (Saha *et al.*, 2020). The pH/EC/TDS/Temperature meter (HANNA-H19813-6 with CAL check ON, Canada) was used. 20 g of an air-dried soil sample from RD and RD-based amendment were diluted in 40 mL of de-ionized water (1:2 ratio) in 50 mL polypropylene tubes (VWR, Mississauga, ON, Canada). Each of the samples was stirred for 1 h at 100 RPM. Samples were left to settle for 30 min, and the pH the supernatant of the solution was measured.

2.2.7 Mineral composition of rock dust and rock dust-based amendments

Four replications of the formulated RD-based amendments were sent to the Department of Fisheries, Forestry, and Agriculture laboratory in St. John's for analysis. Below is the method used to analyze the mineral composition of the media amendments.

2.2.7.1 Sample preparation for media formulation

Soil samples were placed in drying ovens at 40°C and left until fully dry (maximum drying time would be one week). Once dry, samples were sieved under a fume hood using 2.00 mm sieves.

2.2.7.2 Sample analysis

Each sample's pH reading was recorded using a 1:1 ratio with deionized water and the Fisher Scientific Accumet XL250. If the pH reading was less than 6.4, a buffer pH reading was taken using Adams-Evans Buffer solution. Organic matter percentage was calculated using the loss on ignition method. The sample was placed into a weighed crucible left to dry overnight in a drying oven set at 105 °C. Afterward, allowed to cool and weigh the crucible. Then, the sample was placed in a muffle furnace at 430 °C for 6 h. When the crucible was cooled, it was weighed again; the soil sample extraction was done using Mehlich-3 for element analysis using pre-made stock solutions. The filtered samples were read on an auto-analyzer (Prodigy inductively coupled plasma (ICP-OES) spectrometer) following the method of (Simard, 1993).

2.2.7.3 Active microbial community analysis

Fatty acids as methyl esters were analyzed by gas chromatography-mass spectrometry with flame-ionization detection (GC-MS/FID) using the PLFA(phospholipid fatty acid analysis) platform to determine the active microbial community structure in the individual media formulations according to the method described by Zaeem *et al.* (2019). The retention times and the mass spectra of each fatty acid methyl esters (FAMEs) identified were compared with those obtained from known standard mixtures or pure PLFAs (Lazcano et al., 2013). The standards were supplied by Larodan Lipids (Malmö, Sweden) for a calibration curve for each FAME, including those from the cis and trans form of PLFA 18:2ω6 and subsequently, to identify them by GC-MS analysis. Certain PLFAs were used as biomarkers to determine the presence and abundance of specific microbial groups (Zelles, 1999). The PLFAs considered mainly of bacterial origin were further classified as Gram-positive bacterial PLFAs and Gram-negative bacterial PLFAs. The PLFA 10 Me18:0 was used as a biomarker for actinomycetes bacteria, and the PLFAs 18:2ω6c and 18:1ω9c were used as fungal biomarkers (Lazcano *et al.*, 2013).

2.2.8 Statistical analyses

Data were statistically analyzed by one-way analysis of variance (ANOVA) using the XLSTAT Premium Version (Addinsoft, New York, USA) program (Zaeem *et al.*, 2019). The differences between treatment means were separated by Fisher's Least significant difference (LSD; $\alpha = 0.05$). Pearson's correlation analysis was performed to test the relationships between variables. Multivariate analysis such as principal component analysis (PCA) was performed to determine the effect of RD and media amendments on physicochemical properties of plant growth media, nutrient, and active microbial community, and their relationship. to complement the ANOVA.

2.3 Results and discussion

2.3.1 Metal of environmental concern of rock dust

Heavy metals pose severe environmental issues, including risks and hazards to animals and human health. They can be released into the soil by anthropogenic activities such as mining by-product disposal (Tchounwou *et al.*, 2012)**.** The most common heavy metals are copper, nickel, zinc, cadmium, lead, chromium, arsenic, and mercury. These metals are not biodegradable or easily acted upon by microbial activities. Therefore, heavy metals from the soil can pose environmental risks to other ecosystems (Zhu *et al.*, 2021). They could find their way into the human system through direct contact, polluted water, and into the food chain, causing a reduction in food quality.

Current analysis of heavy metal and nutrient composition in RD from the Anaconda mine site has been reported to be made up of an abundance of traces of various elements and very low concentrations of arsenic, lead, mercury, nickel, and natural radioactive heavy elements (Arnott *et al.*, 2021). The concentration of heavy metals in RD was found to be very low(e.g mercury = 0.02 mg/kg^{-1}dry soil) and below the safe limits of the Canadian Council of Ministers of the Environment (CCME) (Environment, 2002*)* for agricultural soils as well as biosolids safe limit (Arnott *et al.*, 2021). The low contaminant level in RD makes it safe to be used as a media amendment for re-mineralizing podzolic soils and the safe growing of crops for human consumption. The safe use of RD has also been reported to be a soil mineralizer that meets the standard required for organic farming (Theodoro *et al.*, 2020).

2.3.2 Particle size distribution of rock dust

A large proportion of the particle size was less than 0.5 mm in diameter (**Fig** 2.1). The particle size was observed to be similar among the three samples and made up of 40% sand, 30% silt, and 30% clay across multiple composite samples taken in transects from different areas of the storage site (**Fig**.2.1). The RD analyses report used in this study showed similar particle size distribution as reported by (Kelland *et al.*, 2020). Studies conducted by Plata *et al.* (2021) reported that the lower particle size distribution might increase the release of nutrients.

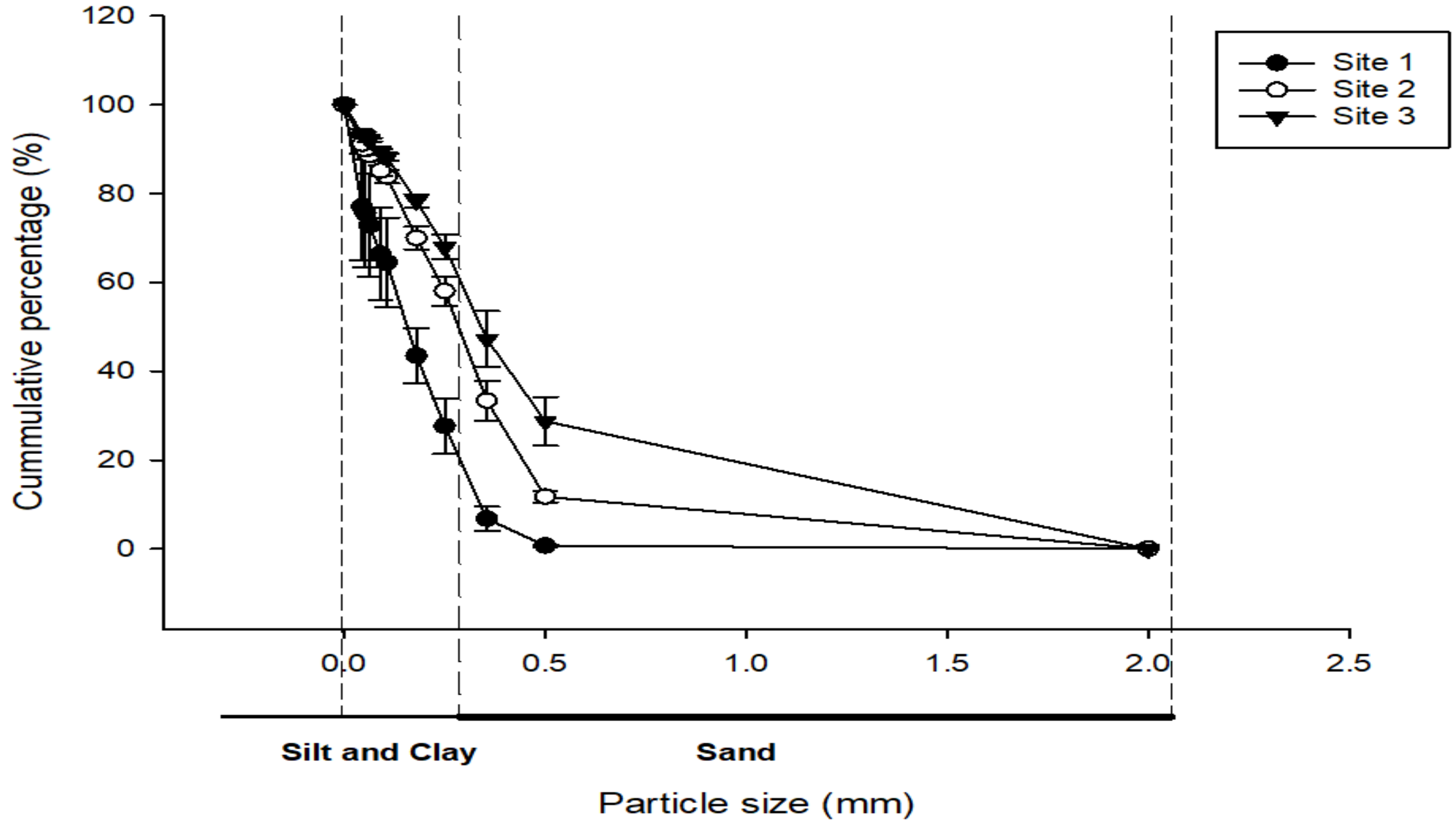

Figure 2.2 . Particle size distribution of RD samples from three Anaconda Mining sites located in Baie Verte, NL. Values on the X-axis represent the cumulative percentage of the particle size, and Y-axis represents the average (n=4) percentage of particle size per site.

2.3.3. Physical characteristics of formulated RD-based amendments

Bulk density has been previously reported to indicate good soil health, which influences the nutrient composition, porosity, water retention, infiltration, root depth/restriction, and microorganisms (Indoria *et al.*, 2020). Field capacity and the porosity in every soil indicate a favorable soil condition and its ability to hold available water and minerals for plant uptake (de Oliveira *et al.*, 2015). For example, the water and air content are ideal for crop growth at field capacity (Rai *et al.*, 2017). Air and water contribute to a suitable physical environment for plant growth. Water and air serve as a medium for temperature balance through evaporation and cooling. The soil water content is essential for plant biochemical reactions in aiding nutrient transportation and circulation of oxygen for plant root development (Ritchie, 1998; Ben-Noah and Friedman, 2018). This study observed changes in the bulk density resulting in low bulk density and increased porosity during the media formulation. The formulated media and RD analysis showed significant differences among the treatments (Table 2.2) concerning bulk density, porosity, and field capacity. Additionally, all the amendments had bulk density below the root-restriction threshold of not more than 1.6g/cm^3. For instance, the following treatments RB, TS, RT, RP, RCP, and the control P, showed the lowest bulk density compared to the other treatments (RBP, RDT, H, RD) (Table 2). The amendment with 50% Promix^{TM} to 50% RD had a bulk density of 0.87 g/cm^3, the addition of 50% RD to 50% biochar reduced bulk density to 0.96 g/cm^3, a combination of 50% RD and 50% TS lowered bulk density to 1.10 g/cm^3 and lastly, the addition of 25%compost, 25%Promix^{TM} to 50%RD resulted in a low bulk density of 0.93 g/cm^3. Our results agree with Kukal *et al.* (2012), who reported a significant increase in volumetric water retention and increased porosity of organic amendments with soil.

As an example, the improvement in the bulk density of the combination of RD and biochar could be ascribed to the porous structure and large specific area of biochar and RD. These properties can potentially change pore-size distribution, bulk density, and surface area and impact media structure and porosity, as Ni *et al.* (2020) reported. There was an increase in porosity in all the permutations but did not exceed the acceptable water-filled pore space of 60% (Table 2.2). The improved porosity indicates suitable moisture content for soil respiration and nitrogen cycling, enhancing their infiltration level and soil structure properties (Luna *et al.*, 2018).

The present results could be ascribed to the organic matter content of amendments which reduces soil bulk density to permit root growth and enhances micro-porosity in soil (Kukal *et al.*, 2012). The overall result of the bulk density of RD-based amendments was within the accepted range for root growth, as reported by Mukhopadhyay *et al.* (2019). Their experiment results revealed that bulk density less than or equal to 1.3 g/cm^3 was considered good, between 1.3 g/cm^3 and 1.55 g/cm^3 is fair, and greater than 1.8 g/cm^3 is deemed to resist root growth. We could assume that RD and RD-based amendment could be potential /alternative media for crop production.

Table 2.2. The physical composition of rock dust and rock dust-based amendments.

Treatment	Bulk density (mg/cm^3)	Porosity (%)	Field capacity (%)
P	0.24 ± 0.06^a	60.69±0.02^c	30.52±0.04^{bc}
RBP	1.17 ± 0.01^g	44.00±0.01^a	35.90±0.01^c
H	1.19 ± 0.01^f	41.07±0.01^a	22.62±0.01^a
RT	0.85 ± 0.01^c	56.44±0.01^b	35.15±0.01^c
RP	0.87 ± 0.01^c	55.87±0.01^b	36.29±0.01^c
RCP	0.93 ± 0.01^e	59.85±0.02^{bc}	36.09±0.03^c
RD	1.50 ± 0.01^h	43.92±0.02^a	26.74±0.03^c
RDT	1.10 ± 0.01^f	43.21±0.01^a	30.40±0.01^{bc}
RB	0.96 ± 0.01^d	45.74±0.01^a	31.84±0.01^a
TS	0.57 ± 0.00^b	71.66±0.04^d	32.11±0.01^{bc}
Recommended level	<1.6	50-70	10–33.

Values represent means ± standard error. Means in the same row accompanied by different superscripts are significantly different at LSD α=0.05, n=4 per experimental replicate. P = 100%Promix(positive control), RBP = 50%Rock dust + 25%Biochar + 25%Promix. H = Huplaso(negative control), RT = 25%Rock dust + 75%Topsoil, RP = 50%Rock dust + 50%Promix, RCP =50% Rock dust +25% Compost + 25%Promix, RD =100% Rock dust(by-product), RDT = 50%Rock dust+50%Topsoil , RB = 50%Rock dust + 50%Biochar, TS = 100%Topsoil.

2.3.4. Nutrient composition of RD and the formulated amendments

The study demonstrated that RD contains a substantial amount of macro- and micro-nutrients including, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), Sulphur (S), zinc (Zn), copper (Cu), iron (Fe), boron (B), manganese (Mn), and aluminum (Al) (Table 2. 3). The result from the soil analysis (Table 2.3) supports the potential of RD and RD-based amendment to be a suitable growth media amendment to provide plant with adequate nutrients for survival. Previous studies outline the function of the minerals found in RD. For example, Ca and Mg are essential for crop growth and to support enzymes function and nutrient transportation and boron (B) is responsible for cell wall and membrane integrity, and Mg is responsible for photosynthetic activity (Gilliham *et al.*, 2011; Broadley *et al.*, 2012; Guo *et al.*, 2016).

The presence of nutrients in the growth media of the present study could be ascribed to the combination of other amendments (Promix, compost, and biochar) to the already nutrient-rich RD. For example, biochar contains high carbon content, while compost, topsoil, and Promix™ contain high organic matter content (Gould, 2015). These properties influenced nutrient availability through organic matter decay and cation exchange capacity. The findings from this study concur with previous studies conducted by Ramos *et al.* (2015) and Ramos *et al.* (2020a). Their study concentrated on the physical, mineralogical, and chemical characterization of a volcanic-rock mining by-product and the use of black oats and maize trials in the greenhouse to assess the by-product's potential use as a soil remineralizer. The results revealed that the RD amendment contain significant amount of trace minerals and plant nutrients suitable to support plant growth or crop production.

Table 2.3. Quantity of extractable minerals and pH of rock dust and rock dust-based amendments.

Minerals (*mg L^{-1}*)	**RD**	**TS**	**H**	**P**	**RP**	**RB**	**RT**	**RDT**	**RCP**	**RBP**	**Level required for crop growth**[1,2,3]
Phosphorus	1^{a}	109^{f}	4^{ab}	89^{e}	15^{cd}	31^{d}	86^{e}	76^{e}	25^{cd}	15^{bc}	12 - 14
Potassium	35^{a}	201^{c}	101^{b}	387^{e}	254^{d}	13^{a}	44^{a}	29^{a}	152^{b}	116^{b}	121 - 181
Calcium	9747^{f}	3905^{j}	4909^{e}	2435^{a}	11,102^{j}	10,497^{ij}	4,102^{d}	3,029^{bc}	9,470^{f}	10,276^{h}	>40 - 60
Magnesium	126^{b}	175^{e}	517^{h}	217^{g}	205^{f}	132^{c}	130^{b}	104^{a}	217^{i}	169^{d}	50 - 70
Sulphur	40^{g}	40^{g}	34d^{e}	37^{f}	33de	22^{a}	30^{c}	26^{b}	31^{cd}	37^{ef}	10 - 20
Zinc	0.4^{a}	20.2^{i}	1.6^{d}	1.4^{cd}	2.9^{f}	2.1^{ef}	12.6^{h}	7.8^{g}	1.3^{c}	0.9^{b}	>1.5
Copper	1.6^{c}	2.7^{d}	0.9a^{b}	0.6^{a}	2.7^{de}	2.7^{de}	2.8^{e}	3.2^{f}	0.9^{ab}	1.1^{b}	1 -1.8
Sodium	11^{a}	110^{f}	114^{g}	86^{e}	45^{c}	78^{d}	11^{a}	75^{d}	21^{b}	21^{b}	-
Iron	396^{c}	236^{b}	1175^{d}	73^{a}	259^{b}	241^{b}	248^{b}	258^{b}	249^{b}	255^{b}	6.0 - 1x10^{6}
Boron	1.2^{c}	1.7^{d}	4.3^{e}	0.3^{a}	0.5^{ab}	0.4^{ab}	0.9^{bc}	0.6^{ab}	0.3^{a}	0.3^{bc}	1 - 3
Manganese	147^{f}	73^{c}	77^{d}	6^{a}	174^{j}	163^{h}	72^{b}	83^{e}	148^{g}	166^{h}	20 - 30
Aluminum	38^{b}	439^{d}	626^{e}	41^{b}	7^{a}	24^{ab}	435^{d}	228^{c}	12^{a}	17^{ab}	<2 - 5
pH	*8.4^{de}*	*7.2^{f}*	*9.1^{g}*	*4.9^{a}*	*7.4^{c}*	*8.0^{de}*	*8^{ef}*	*7.1^{ef}*	*6.5^{b}*	*7.4^{cd}*	*5.5-7.0*

Mineral composition and pH level of rock dust, rock dust-based amendment, Topsoil, compost and Promix™. The last Column shows the required level of nutrient and pH for crop growth. P = 100%Promix(positive control), RBP = 50%Rock dust + 25%Biochar + 25%Promix. H = Huplaso(negative control), RT = 25%Rock dust + 75%Topsoil, RP = 50%Rock dust + 50%Promix, RCP =50% Rock dust +25% Compost + 25%Promix, RD =100% Rock dust(by-product), RDT = 50%Rock dust+50%Topsoil , RB = 50%Rock dust + 50%Biochar, TS = 100%Topsoil. Means in the same row accompanied by different letters are significantly different at LSD α=0.05, n=4 per experimental replicate.

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2.3.5. Microbial Community

Soil microbial communities enhance soil ecological and economic importance (Singh *et al.*, 2009; Strickland *et al.*, 2009; Bodelier, 2011; Philippot *et al.*, 2013). To the best of our knowledge, there is not much literature information on the microbial community in RD to support its potential as a media amendment for crop production.

Phospholipid fatty acid (PLFA) analyses were conducted to investigate the active microbial community in RD and RD-based amendment to provide the qualitative and quantitative microbial community composition. PLFA is a component of microbial organisms' membrane that is used to verify the presence of different classes of soil microbes to provide empirical information regarding their characteristics such as total biomass, physiology, taxonomy, and overall community composition.

Our findings revealed that RD and RD-based amendments contain a significant amount of diverse, active microbial biomass (Table 2.4). The bacterial community was substantial compared to fungi and protozoa in all the treatments except huplaso, which shows a higher fungi population. There was a significant effect of microbial composition among the treatments. G+ and G- were the highest across treatments, while eukaryotes, protozoa, and fungi were the least. The study observed diverse, active microbial communities in the RD-based amendments. The following growing media, RB, RT, RDT, and RCP, showed a significant increase in G- and G+ bacterial community compared to the control P. The diversity of microbial communities could be attributed to different combinations of media with different physicochemical properties to provide favorable conditions for the growth of the active microbial community (Nett *et al.*, 2012). Biochar, for example, may improve soil physical structure, including aeration, increase soil nutrient concentrations and pH, and provide macroporous environments that promote microbial colonization (McCormack *et al.*, 2013; Gul *et al.*, 2015). These observations agree with the experiment conducted by Koyama *et al.* (2014), who revealed that soil microbial composition was influenced by increased nutrient availability in Arctic tundra soils. The present investigation demonstrated a significant increase in G-&G+&F in RD-based amendment RBP (28.38±4.88%) as compared to the control P (9.49±0.06%) (Table 2.4). The high microbial composition of the RD-based amendments could be ascribed to the clay minerals, organic carbon content, and organic matter supplied by the mixture of RD, biochar, and Promix^{TM}, respectively.

Two-dimension principal component analysis (PCA) conducted on the media amendments and the soil microbial community data explained 85.52% of the total variation in the data set. PC1 and PC2 displayed 60.95% and 24.57% of the total variability, respectively (Fig 2.2). The observation plot depicted distinct segregation of the media amendment based on the centroid where RDT and RB amendments were grouped in quadrant 4 of the observation plot (Fig 2.2A). Media RBP and H are clustered in quadrant Q-1, TS, RD, P, RCP, RT RP, and P clustered in quadrant Q-4.

The biplot displays the relationship among the different variables where eukaryotes and G-, G + & G- bacterial grouped with RDT and RB growth media. The presence of microbial community in RD and RD-based amendment could indicate good soil health, as stated by Mathew *et al.* (2012). The findings from the present study demonstrated that RD-based amendments promoted the growth and diversification of active microbial communities, possibly serving as a good indicator of RD-based media quality and potential as a suitable media amendment for crop growth.

Table 2.43: The microbial composition of rock dust and rock dust-based media amendments.

Treatment	Eukaryotes (%)	G- (%)	G+ (%)	F (%)	P (%)	G+ & G- (%)	G+&G-&F (%)
P	3.52±0.09^{d}	21.72±0.39^{de}f	26.40±0.25^{def}	9.33±0.13^{c}	3.30±0.08^{e}	26.25±0.02^{b}	9.49±0.06^{c}
RBP	4.94±0.35^{f}	18.45±0.93^{b}	9.71±0.73^{ab}	7.12±0.57^{b}	1.44±0.11^{ab}	29.97±2.21^{c}	28.36±4.88^{d}
H	0.00±0.00^{a}	2.99±0.25^{a}	7.51±0.05^{a}	40.16±0.13^{g}	0.77±0.03^{a}	18.33±0.16^{a}	30.49±0.16^{e}
RT	1.37±0.03^{b}	19.91±0.38^{bc}	22.48±0.11^{d}	11.76±0.17^{f}	2.55±0.07^{ab}	36.06±0.26^{e}	5.89±0.17^{ab}
RP	4.21±0.10^{e}	23.65±1.15^{d}	27.24±0.36^{e}f	10.01±0.23^{cde}	1.98±0.09^{bc}	25.43±0.07^{b}	7.49±0.17^{abc}
RCP	2.15±0.08^{c}	19.89±0.23^{bc}	23.28±0.41^{de}	12.67±0.06^{f}	2.68±0.10^{d}	31.81±0.41^{cd}	7.52±0.07^{ab}
RD	1.98±0.11^{bc}	22.00±1.09^{cd}	26.39±0.39^{def}	9.45±0.32^{cd}	2.79±0.25^{de}	24.49±0.52^{b}	12.91±2.49^{c}
RDT	8.27±0.45^{g}	31.16±1.33^{e}	17.00±3.97^{c}	5.21±0.39^{a}	1.42±0.19^{ab}	34.15±1.55^{de}	2.78±0.20^{a}
RB	3.97±0.11^{de}	23.70±1.10^{d}	12.01±0.31^{b}	10.38±0.13^{de}	1.41±0.08^{ab}	36.91±0.46^{e}	11.61±0.12^{bc}
TS	1.67±0.04^{bc}	17.78±1.13^{b}	30.19±0.46^{f}	10.44±0.20^{e}	2.30±0.05^{cd}	31.38±0.49^{cd}	6.25±0.02^{ab}

Different letters show significant differences between treatments. Means in the same row accompanied by different superscript are significantly different at LSD α=0.05, n=4 per experimental replicate. P = *100%*Promix(positive control), RBP = *50%*Rock dust + *25%*Biochar + *25%*Promix. H = Huplaso(negative control), RT = *25%*Rock dust + *75%*Topsoil*,* RP = *50%*Rock dust + *50%*Promix, RCP =*50%* Rock dust +*25%* Compost + *25%*Promix, RD =*100%* Rock dust(by-product), RDT = *50%*Rock dust+*50%*Topsoil , RB = *50%*Rock dust + *50%*Biochar*,* TS = *100%*Topsoil

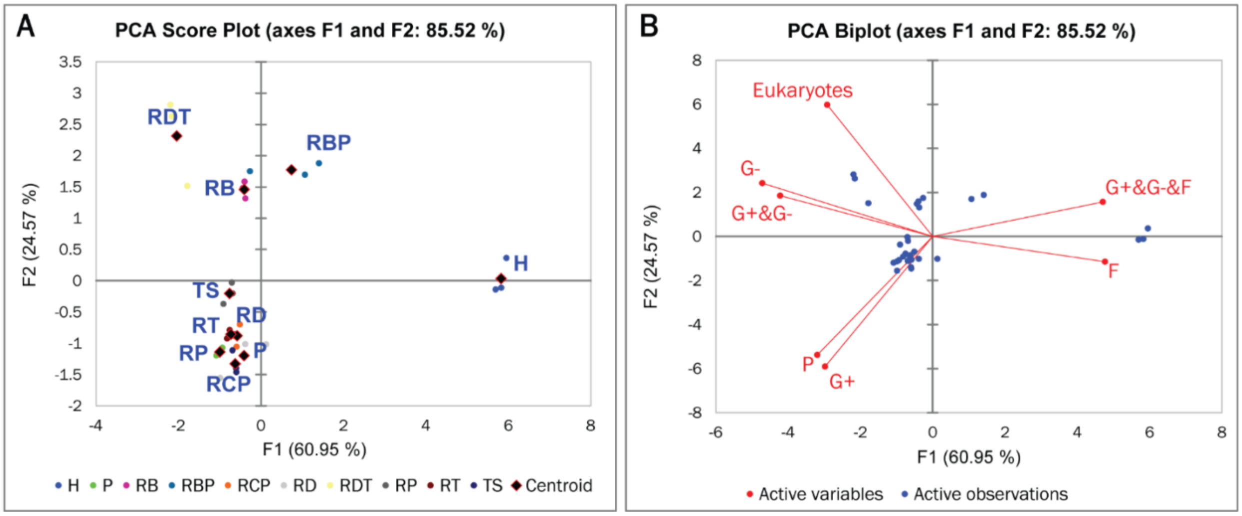

Figure 2.2. (A) Score plot and (B) Biplot from principal component analysis (PCA) showing clusters of the rock dust-based media amendments based on the soil microbial community composition. Centroids of the 10 media formulations are indicated by diamonds, n=4 per treatment. P = 100%Promix(positive control), RBP = 50%Rock dust + 25%Biochar + 25%Promix. H = Huplaso(negative control), RT = 25%Rock dust + 75%Topsoil, RP = 50%Rock dust + 50%Promix, RCP =50% Rock dust +25% Compost + 25%Promix, RD =100% Rock dust(by-product), RDT = 50%Rock dust+50%Topsoil , RB = 50%Rock dust + 50%Biochar, TS = 100%Topsoil.

2.3.6. Relationship between rock dust media amendments, physical properties, nutrient composition, and microbial biomass

Our results from the (PCA) revealed the relationship between RD's physical properties, RD-based amendment, and microbial community (Fig 2.3). The PCA explained 63.44% of the total variability in the data set where component 1 displayed 41.39%, and component 2 showed 22.06% variation, respectively. The following treatments RB, RD, RP, and RCP were clustered in quadrant 4 of the PCA RDT, RT, P, and TS, and RT were clustered in quadrant 3, RBP, and H separated in quadrant 1 and 2, respectively (Fig 2.3). However, the control clustered with other treatment in the lower quadrant but the negative control segregated in the lower quadrant of the PCA. Treatments TS and P were grouped in quadrant 1, while treatment H separated from all other treatments into quadrant 2.

Pearson correlation analysis was conducted to understand the above relationship better, and the results revealed a strong negative correlation (r=**-0.982)** of G+ &G-&F with soil pH. However, we observed a positive correlation (r=**0.62**) of mineral Fe with G+ &G-&F. There was also a strong positive correlation of soil porosity with G+(gram-positive bacteria) (r=**0.72**) and (r=**0.63**) with Zn. We observed a strong positive correlation (r=**0.85**) of Ca with eukaryotes and gram-negative bacteria. At the same time, Potassium(K) showed a strong positive correlation (r=**0.58**) with Gram-positive bacteria and protozoa (*1P*) (r=**0.74**) (Table 2.5).

The result from the present study improves our understanding of the relationship of RD-based amendments with soil microbial composition, physicochemical properties, and nutrient availability, influencing the overall quality of media. Previous studies have revealed that the soil's physicochemical properties influence the microbial structure of the soil (Lauber *et al.*, 2008). Soil properties have been reported to impact the microbial community, directly and indirectly, impacting plant abiotic and biotic stress via microbial diversity, composition, and network interactions (Yang *et al.*, 2017).

Furthermore, Yang *et al*. (2019) demonstrated that soil aggregates of 2–4 mm and 1–2 mm had high respiration and porosity, respectively, influencing soil microbial community. A closer look at the active microbial community in this study revealed that RD-based amendment influenced microbial composition. Conversely, the microbial composition of the media amendments influenced mineral disaggregation and dissolution by excreting organic acids and carbon dioxide, which react with water to generate carbonic acid, to dissolve calcium-rich rocks slowly to release nutrients available in RD, as reported by (Ribeiro *et al.*, 2020). The positive correlation of the media nutrient composition (Ca, Zn, Fe) with the microbial composition suggests that RD-based amendment could create an environment favorable for active soil microbes, which mineralized these elements from the rock dust-based media amendment. Overall, the RD-based media formulated appears to have the suitable quality to support soil microbial growth that enhances nutrient mobilization and availability that maybe be adequate for plant growth, development, or crop production.

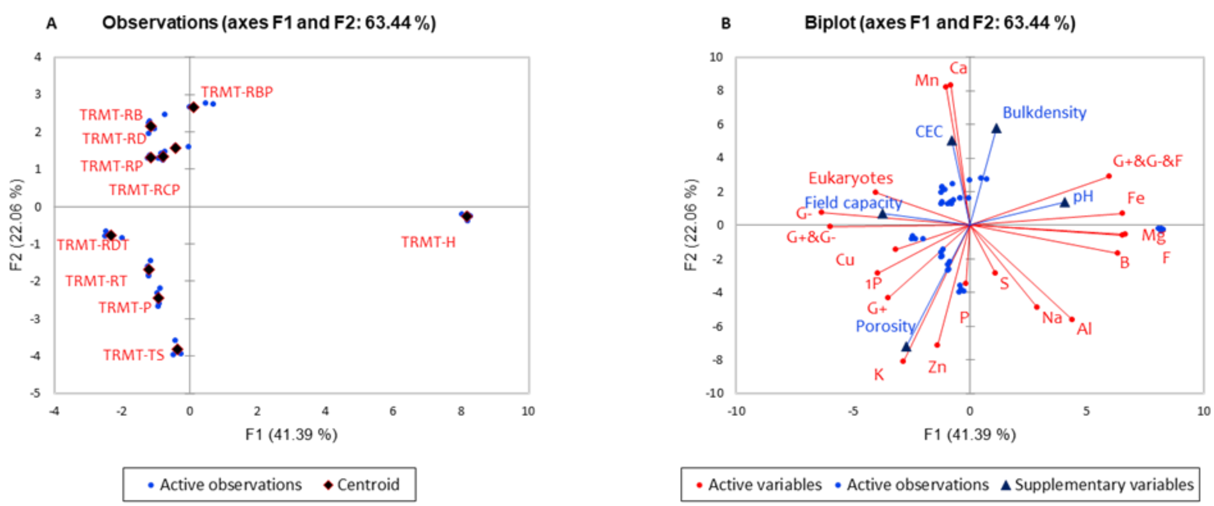

Figure 2.3: Principal component analysis (PCA) of physical and chemical composition of rock dust-based amendment (A) Observation (B)Biplot. P = 100%Promix(positive control), RBP = 50%Rock dust + 25%Biochar + 25%Promix. H = Huplaso(negative control), RT = 25%Rock dust + 75%Topsoil, RP = 50%Rock dust + 50%Promix, RCP =50% Rock dust +25% Compost + 25%Promix, RD =100% Rock dust(by-product), RDT = 50%Rock dust+50%Topsoil , RB = 50%Rock dust + 50%Biochar, TS = 100%Topsoil.

Table 2.5: Correlation between physicochemical and microbial properties of rock dust amended media.

QUADRANT 1 (RBP)	**Variables**	*pH*	*G+&G-&F*				
	pH		**-0.982**				
	G+&G-&F	**-0.982**					
	Fe		**0.618**				
QUADRANT 2 (H)	**Variables**	*F*	*Na*				
	Na	**-0.99**					
	Al		**0.958**				
QUADRANT 3 (RDT, RT, P, TS)	**Variables**	*Porosity*	*G+*	*1P*	*G+&G-*	*K*	*Zn*
	G+	**0.715**					
	G+&G-		**-0.574**	**-0.575**			
	P	**0.680**					
	K		**0.580**	**0.737**	**-0.885**		
	Zn	**0.634**					
	Cu			**-0.822**	**0.876**	**-0.902**	**0.558**
QUADRANT 4 (RB, RD, RP, RCP)	**Variables**	*CEC*	*Eukaryotes*	*G-*			
	G-		**0.585**				
	G+	**0.949**					
	Ca		**0.847**	**0.579**			

The media compositions are in italics :(Bulk density, Porosity, Field capacity ,Eukaryotes, G-=negative, G+=gram positive ,F=fungi, 1P=protozoa, G+&G-=gram positive and negative bacterial, pH=soil pH, CEC=cation exchange capacity P=Phosphorus, K=Potassium, Ca=Calcium, Mg=Magnesium S=Sulphur , Zn=Zinc, Cu=Copper Na=Sodium, Fe=Iron, B=Boron, Mn=Manganese, Al=Aluminum).. P = 100%Promix(positive control), RBP = 50%Rock dust + 25%Biochar + 25%Promix. H = Huplaso(negative control), RT = 25%Rock dust + 75%Topsoil, RP = 50%Rock dust + 50%Promix, RCP =50% Rock dust +25% Compost + 25%Promix, RD =100% Rock dust(by-product), RDT = 50%Rock dust+50%Topsoil , RB = 50%Rock dust + 50%Biochar, TS = 100%Topsoil. n=4, Values in bold are different from 0 with a significance level alpha=0.05

2.4.0 Conclusion

Our research has demonstrated that RD particles are below 2 mm diameter with a particle size distribution of 40% sand, 30% clay, and 30% silt.

RD has a similar particle size as clay and silt, demonstrating the ability to store and hold cations which support the enhanced availability of nutrients and fertility of RD amended media. To confirm the above indications, analysis of the RD samples revealed the availability of several nutrients essential for crop growth and production. For example, a high amount of calcium (Ca) was observed in the following media formulation: RP>RB>RD=RCP, while we also observed a high amount of Mg in the subsequent media amendments RP>RBP>RCP>RD. The active microbial community found in RD-based amendments appears to be responsible for the weathering of RD to release the nutrients available for plant growth. For example, strong significant and positive correlations were observed between Fe, K, Ca, and G-&G+&F. Furthermore, RD-based amendments were found to have low bulk density and high porosity, which indicates a potential for retaining moisture content for soil respiration and nitrogen cycling due to enhancing their infiltration level and allowing root development of plants.

Results depicted that RD amendments in different growth media resulted in improved growth media quality, including lower bulk density, higher porosity, improved soil microbial communities, and nutrient status compared to non-RD amendments. Overall, the study demonstrated the potential of RD as a suitable media amendment for the production of vegetable crops under controlled environmental conditions. This could be a strategy used to provide a sustainable solution for the by-product disposal and serve as an alternative soil mineralizer in the horticultural industry.

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CHAPTER 3

Assessment of the media quality on the agronomic performance and nutritional quality of horticulture crops cultivated in rock dust based natural media amendment in controlled environmental conditions

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Abstract

Rock dust (RD) is a by-product of mineral mining with limited commercial applications and is traditionally discarded as industrial waste. The chemical composition of RD appears to have promising potential as a soil media amendment for horticultural crops production under controlled environmental conditions. This study was designed to assess RD's effectiveness as a soil amendment and as well as determine its effects on the agronomic performance and nutritional quality of three horticultural crops cultivated in a controlled environment. The RD was tested against available market growth media amendments and in different combinations with soil. There were a total of ten growth media formulations including: 1) 100%Rock dust (RD), 2) 50%Rock dust+50% Topsoil(RDT), 3) 50%Rock dust+ 25%Biochar + 25% Promix (RBP) 4) 100%Topsoil (TS) ,5) 25%Rock dust + 75% Topsoil (RT), 6) Huplaso (negative control), 7) 50%Rock dust+ 25% compost +25% Promix (RCP), 8)50%RD+ 50%Promix (RP), 9) Promix(P) (Control), 10) 50%Rock dust + 50%biochar (RB). Two crop cycles were completed, and the experiments were carried out in a completely randomized design with four replicates in the growth chamber. Crop agronomic performance and produce nutritional quality were tested in kale, amaranth, and lettuce. The media amendments showed significant effects on plant biomass and quality parameters in three crops. RD in the media resulted in a significantly higher plant root-shoot ratio in lettuce and amaranth. RCP, RBP, and control P showed a significant increase($p<0.05$) in total biomass (TBM), and the number of leaves, in both lettuce and kale over other treatments. The protein content of the amaranth crop was enhanced significantly when grown in RCP compared with the control P. The total antioxidant content of lettuce analyzed with ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) showed a significant increase in the following treatments RT>RCP>RD over the control P. The color of steamed amaranth was rated highest, while lettuce crops grown in RB were ranked highest for overall liking and appearance. This was supported by the strong positive correlation between BD (Bulk density) and total macro minerals, a negative correlation of BD with G+&G-&F, and a strong positive correlation of porosity with FW (Fresh weight) and TBM (total biomass). This study demonstrated an increase in total macro minerals in plant tissue, FW, TBM, MUFA (Monosaturated fatty acid), increased protein content, and total antioxidants. This is supported by the strong association observed between the media quality and the agronomic performance as well as the nutritional composition. The strong association suggests that RD-based media have superior bulk density, porosity, nutrient composition, and microbial composition in enhancing the growth and phytochemical composition of vegetable crops. These results suggest that RD-based amendments (RCP, RBP, RB, and RP) could be used as a suitable media amendment and a sustainable method of providing mineral nutrients to crops for optimum growth and productivity geared towards improving food security and environmental sustainability. Further optimization of the above media would be needed for recommendations to farmers.

Keywords: Mine tailing waste, soil amendment, plant growth, vegetable quality, phytochemical, antioxidant, vitamins.

3.1 Introduction

Waste products generated through mining activities can cause a significant loss of biodiversity, air quality, and water pollution affecting humans, plants, and animals through soil degradation (Mugica-Alvarez *et al.*, 2015). Despite the significant contributions to society, mining activities have become a severe threat to biodiversity and food security (Christou *et al.*, 2017). For instance, some mining activities adversely affect the ecosystem functioning, including the loss of arable land, soil degradation, polluted air, and reduced water quality, leading to declining agricultural productivity, food insecurity, and economic growth (Martínez-Sánchez *et al.*, 2012).

In the mining and quarrying industries, RD is a mineral-rich by-product that can be used to improve soil fertility, promote plant growth, raise the activity of beneficial microflora, increase pest and disease resistance, as well as enhance the quality of fruits and vegetables (Beerling *et al.*, 2018). According to Li and Dong (2013), the nutritional content of RD could be used as an amendment to improve soil fertility, particularly in nutrient-poor soils. RD contains calcium and trace elements such as iron (Fe), aluminum (Al), and magnesium (Mg) but lack a substantial amount of nitrogen (N), phosphorus (P) and potassium (K)

RD amendment has been reported to be a suitable medium for crop production due to its ability to improve soil water retention, soil pH, electric conductivity, microbial community, and nutrient for crop root uptake (Mathew *et al.*, 2012; Plata *et al.*, 2021). In soil media formulation, different organic amendments such as biochar, vermicompost, peat, and compost were used as media amendments for crop production(Abbey *et al.*, 2019). Previous researches have been conducted across the globe to explore the potential of RD. Fyfe *et al.* (2006) conducted an experiment in Brazil with RD as soil amendment where they compared RD and NPK applications to produce watermelon, rice, cassava, and maize and observed a significant increase in the yield following RD application of up to 40% compared to the plot applied with NPK.

Li *et al.* (2020) evaluated the effect of the rock-compost amendment on soil properties, soil microbial action, and yield. The amendment with RD significantly increased the following nutrient levels: Ca, Mg, Mn, Zn, B, and Al without increasing the phytotoxicity of the crops cultivated with the RD-compost. Also, the amendment had a higher metabolic activity, variety of microorganisms, and an increase of 187% in yield, in the second year contrasted with the untreated control.

Furthermore, Iheshiulo *et al.* (2017) conducted a greenhouse experiment with kale grown in different media amendments, such as volcanic RD (100g RD)+ 1kg Pomix,50g dry vermicompost +1 kg Promix,47.5g K humate + 1kg Promix, and Promix alone as a control. The results revealed a higher fresh weight of kale grown in the RD amendment compared with the control and the other amendments. Potassium humate and volcanic minerals had greater SPAD (Soil Plant Analysis Development) values than the other treatments. Utilizing industrial by-products such as RD as amendments to improve the nutrient status and health of agricultural lands or supply potting media for crop production in controlled environmental conditions has gained a lot of recent interest in the scientific community (Basak *et al.*, 2021).

Although there is knowledge about RD as a media amendment, very little is known about the effects of RD as a growing media amendment on the soil physicochemical and physical properties and how these mediate the phytochemical properties and nutritional profiles of horticultural crops. We hypothesized that RD from Anaconda mining operation would be a valuable media amendment for quality horticultural crop production due to its nutrient content, low contaminant levels, and enhanced microbial activities, potentially creating commercial use for this mining waste by-product

To test this hypothesis, we formulated seven media amendments with RD and compared them with three controls (Huplaso, Promix, and Topsoil) for growing kale, amaranth, and lettuce. Our specific objectives were: To evaluate growth, yield, nutritional composition which include antioxidant (Antioxidants help protect the human body by preventing the oxidation of biomolecules such as DNA, lipids, and protein (Griñan-Lison *et al.*, 2021)).Finally we evaluated the mineral composition of the crops cultivated to understand better the relationship between the media quality and the above nutritional and agronomic parameters.

3.2 Material and Methods

3.2.1 Experimental setup and plant material

A two-year study was conducted in a walk-in growth chamber (Bio Chambers MB, CA) at the Grenfell Campus of Memorial University of Newfoundland and Labrador Canada (48° 56' 24.32" N, -57° 55' 55.92" W). The experiments comprised three horticultural crops and ten soil media amendments arranged in a completely randomized design with four replications for each crop.

The crop growth conditions were maintained throughout the experiments. Lettuce, amaranth, and kale were grown at 25°c day/ 18°c night temperature with 72% relative humidity. The light intensity was set at 2000- to 4000 lumens with a 16 h day/ 8 h night lighting period. The seeds were purchased from Vesey's Seeds Ltd (York, PE, CA).

3.2.2 Soil media amendments

There were ten different potting media treatments composed of Promix, Biochar, Compost, Huplso, RD, and Topsoil in various combinations, as shown in (Table 3.1**)**. The promix potting media was obtained from Premier Horticulture Inc. (Quakertown, PA, USA). The promix is composed of 75–85% sphagnum peat moss, vermiculite, wetting agent, dolomitic, horticultural grade perlite, and calcitic limestone. The Topsoil, Compost, Huplaso, and pots were obtained from

St-Isidore Asphalte Ltée, Saint-Isidore, NB, Canada. The mine tailing waste RD was obtained from Anaconda Mining Inc. located in the Baie Verte Mining District (49°57′42″N, 56°07′23″W) in north-central Newfoundland, Canada. The RD was sampled from different storage sites and was analyzed for particle size distribution, bulk density, field capacity, and porosity. These analyses were used to facilitate the media formulation and match soil chemistry, structure, and texture for crop growth and development under controlled environmental conditions. Once growth media were formulated, lab analyses were carried out at Soil, Plant, and Feed Laboratory in St. Johns, Newfoundland. The media amendments were made up based on volume (**Table 3.1**). The mixing was done by hand to get a uniform mixing of each treatment using a volumetric flask. There were three controls and seven media formulated, which were transferred individually into 12.95 cm x 13 cm diameter planting plastic pots. As described in (chapter 2) of this thesis.

Huplaso is high-quality volcanic RD media sold on the commercial market and shown to have excellent quality in improving crop growth and productivity. TS is the standard substrate used for crop growth and performance with adequate fertility. TS is universally accepted as a suitable medium for high-quality crop production.

Table 3.1: Different growth media formulations evaluated during this study.

Media treatment	Treatment details
RD	100% RD
P	100% Promix
TS	100%Topsoil
H	100% Huplaso
RDT	50% RD+50%TS
RCP	50%RD+25%Compost+25%Promix
RP	50%RD+50%Promix
RB	50%RD+50%Biochar
RBP	50%RD+25%Biochar+25%Promix
RT	25%RD+75%Topsoil

*3.2.*3 Crop growth during the experiment

Before the start of experiments in each growth cycle, the seedlings of each crop were raised in plug trays for five weeks until they reached two to three true leaves stages. The seedlings were then transplanted into the pots with different soil amendments as one seedling per pot. Pots were irrigated with the fertigation management system twice a day during morning and evening. In order to provide essential nutrients, a stock solution was prepared according to the Hoagland recipe (Hoagland, 1944). Briefly, the nutrient solution were prepared by mixing the following element potassium nitrate (KNO_3) =1.5kg/10L, calcium nitrate($Ca(NO_3)_2$)=2kg/10L, monopotassium phosphate(KH2PO4)=0.75kg/10L, magnesium nitrate($Mg(NO_3)_2$)=1kg/10L ,and iron chelate 0.05kg/10L .The iron chelate consists of three components: Fe3+ ions A complex, such as EDTA, DTPA, EDDHA, amino acids, humic-fulvic acids, citrate. Sodium (Na+) or ammonium (NH4+) ions.

3.2.4 Plant agronomic performance evaluations

Plant agronomic performance was evaluated in all three crops during both crop cycles. The agronomic performance included chlorophyll contents, leaf gas exchange, plant height, number of leaves, root shoot ratio, fresh and dry biomass. The leaf chlorophyll content was recorded using a portable chlorophyll meter (SPAD-502, Minolta Co. Ltd., Osaka, Japan). Chlorophyll readings were recorded from the top three leaves based on the apex of a fully expanded leaf to obtain the optimum chlorophyll content. A portable photosynthesis system (Li6400XT, LI-COR Environmental, Lincoln, NE, USA) was used to measure the photosynthetic rate, stomatal conductance (gs), and transpiration rate (T) in all three crop plants. Measurements were taken between 12:00 - 14:00 with instrument conditions as temperature 25 °c, relative humidity of 53–75%, and carbon dioxide (CO_2) influx at 400 ppm (Yang *et al.*, 2019). Plant height was taken from the surface of the growing media to the leaf tip of the longest leaf of each plant with a measuring tape at harvest (The height of the plant in its natural state). Plant leaf was manually counted to give the total number of leaves in each crop during both crop cycles. The root of the plant was extracted by carefully soaking the root with the bulk soil in a bucket of water to separate the soil from the roots. The water was sieved with a 2mm sieve to collect any detached roots. An electronic balance was used to measure the fresh and dried weight of leaves and roots during both crop cycles. The dry weight was obtained by drying the shoot in a forced-air oven (SL Shel Lab.) at 65°C and weighed with an electronic balance after cooling. The total biomass was determined by the sum of the fresh root weight and the sum of fresh shoot weight.

*3.2.*5 Sensory evaluation

A sensory evaluation test was performed on steamed amaranth and unharvested lettuce crop. Memorial University of Newfoundland (MUN), Grenfell Campus Research Ethics Board approved the procedures for use for the sensory panel evaluations. Consumer panels were conducted at Memorial University of Newfoundland, Grenfell campus. A total of eighty (80) participants were recruited from the community, university students and staff who have no prior knowledge of the crops cultivated.

Selected crop from each treatment was presented to the panelists in random order in a computerized boot. Each product's acceptance and preference were scored on a hedonic structure scale (Vidal *et al.*, 2020) assigned using the sensory analysis software (SIMs 2000). A similar test was done using trained and untrained panelists of 15-20 in-house individuals (for sensory food evaluation). The age range of our participants was 18–59 years. Panelists were asked to rank the crop based on colour, aroma, texture, taste, firmness, appearance, and preference.

Panelists were instructed to fill out each sample as accurately as possible according to their taste experience on a structured hedonic scale.

***3.2.6*. Antioxidant analysis**

The preparation of plant leaf extracts was based on our previous method without any modifications by Thomas *et al.* (2010). Briefly, 100 mg of oven-dried powdered plant sample was mixed with 1 mL of HPLC grade acidified ethanol (0.7% o-phosphoric acid), and the mixture was incubated at room temperature in the dark for 10 min. Afterward, the mixture was centrifuged at 5000 xg for 10 min, and the supernatant was carefully removed without disturbing the pellet. Without further dilution, the supernatant was used to determine lipophilic antioxidant activity (LAA) and lipophilic phenolic content (LPC). The pellet was re-suspended in 50 mM sodium phosphate buffer (pH 7.5), vortexed, centrifuged, and the aqueous supernatant pooled as described by (Manful *et al.*, 2020).

This extract was used to determine the hydrophilic antioxidant activity (HAA) and aqueous phenolic content (HPC).

3.2.7 **Ferric reducing antioxidant power (FRAP*)***

The total antioxidant activity (TAA) of the extracts was measured using the ferric reducing antioxidant power (FRAP) following the method of (Manful *et al.*, 2020), with minor modifications. Briefly, 20 µL of each standard or sample was reacted with 180 µL of freshly made FRAP solution (25mL acetate buffer,2.5 ml TPTZ, AND 2.5 ml $FeCl_3 \cdot 6H_2O$) and the resultant mixture was incubated in the dark for 120 min. Absorbance measurements were recorded at 593 nm on a Cytation Imaging microplate reader (BioTek, Vermont, USA). The TAA extracts' values were determined by summing the LAA and HAA antioxidant activity values based on a Trolox standard curve (0.0- 3.5 mM). The results for the plant extracts were expressed in µmol Trolox equivalent /g extract (dry weight).

3.2.8 **Total phenolic content (TPC) analysis**

The TPC of the plant (lettuce, amaranth, and kale) extracts was measured using the Folin-Ciocalteu reagent as described previously by (Thomas *et al.*, 2010) with minor modifications. Thus, 25 µL of each standard or plant extract was mixed with 125 µL of a 10-fold diluted Folin-Ciocalteu reagent in microplate wells. Acidified methanol (50 µL, 0.7% v/v) or sodium phosphate buffer (50 µL, 50 mM, pH 7.5) was added depending on whether the samples analyzed were lipophilic or hydrophilic extracts, respectively. The resultant mixtures were incubated in darkness for 120 mins, and the absorbance was measured at 755 nm on a Cytation Imaging microplate reader. The values for TPC were calculated by summation of the HPC and LPC, and values measured based on the quercetin standard curve. The results were expressed as µmol quercetin equivalent/g extract.

3.2.9 Antioxidant activity with ABTS method

In determining the hydrophilic and lipophilic antioxidant activities, extractions were done using the methods of Arnao et al. (2001) with minor modifications. For the hydrophilic antioxidant activity, 205.8 µL of 25 mM of ABTS, 875 µL of 125 µMol H_2O_2, and 200 µL of 1 mg/mL HRP (Horseradish peroxidase) in 1160 µL of 50mM phosphate buffer (pH=7.5) were mixed in a total volume of 2 mL. Reaction mixture absorbance was monitored at 760 nm in kinetics mode with a Synergy microplate reader (Bio Tek, Fisher Scientific, Mississauga, ON, CA) until it was stable. For the lipophilic antioxidant activity, a reaction mixture containing 160 µL of 25 mM of ABTS[2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)], 480 µL of 125 µMol H_2O_2, and 1000 µL of 1 mg/mL HRP in 360 µL of 0.7% acidified ethanol in a total volume of 2 mL was prepared. As in the hydrophilic analysis, the absorbance was monitored at 760 nM using the Synergy microplate reader until it was stable. A standard stock solution of Trolox (1 mM) in deionized water was serially diluted to prepare the working standards (10-200 µM). For the HAA (Hydrophilic antioxidant activity) and LAA (Lipophilic antioxidant activity) antioxidant activity, 20 µL of each extract or standard was placed in microplate wells with 200 µL of the corresponding reaction mixture, and the absorbance was measured after 5 min at 760 nm. The difference in the absorbance was related to the ABTS radical cation quenched. The values for the TAA were determined by the summation of the HPA (Hydrophilic phenolic activity) and LPA (lipophilic phenolic activity) values. The results were expressed as µM Trolox equivalents/g of the dry plant sample.

3.2.10 Protein analysis

The protein content in the leaves of three vegetable crops, including amaranth, kale, and lettuce, was determined by following the Branford method. Briefly, a 20 mg weight of dry leaf powdered sample was measured with the electronic balance (Radwag, PS6000/C/2, USA, LLC). The 1 mL of sodium phosphate buffer was added to the dry plant sample and vortex for 5 sec. and left for 30 min at room temperature. The extract was remixed for three sec and was centrifuged at 5000 xg for 10 min, and the supernatant was carefully removed without disturbing the pellet. A 10 µL of each sample was pipette into microplate wells. A 300 µL of Coomassie plus the reagent was added to each wall and mixed with the plate shaker for 30 sec and incubated for 10 minutes at room temperature. The absorbance was then measured at 490 nm using a spectrophotometer (Bio Tek, Cytation 3, Bio Tek, Fisher Scientific, Mississauga, ON, CA (**Fig**. 4).

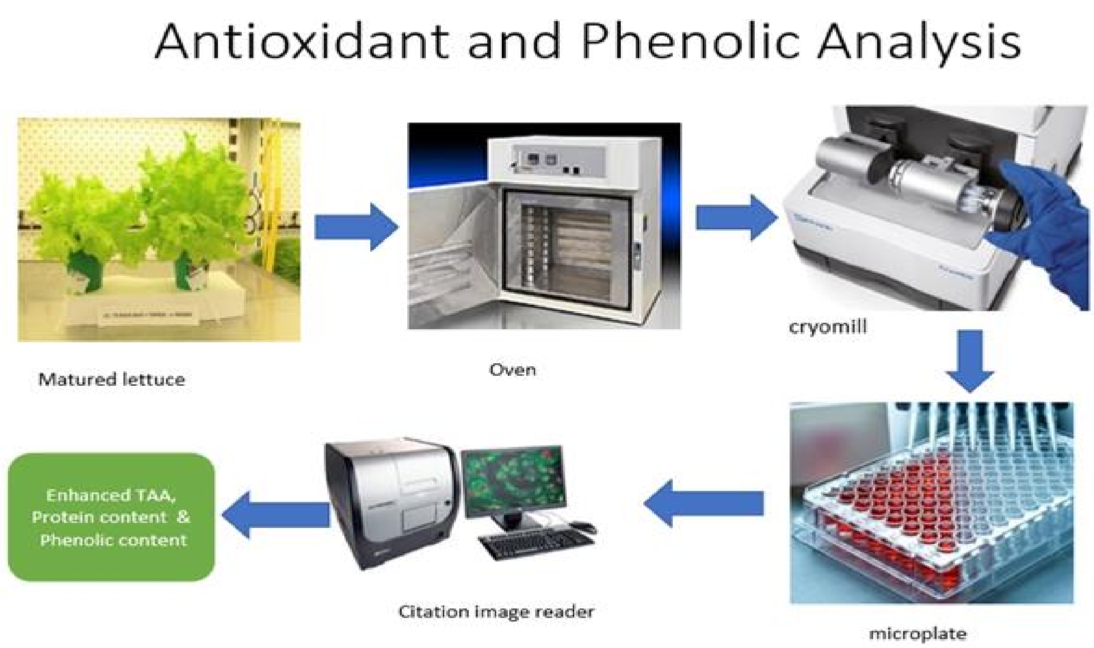
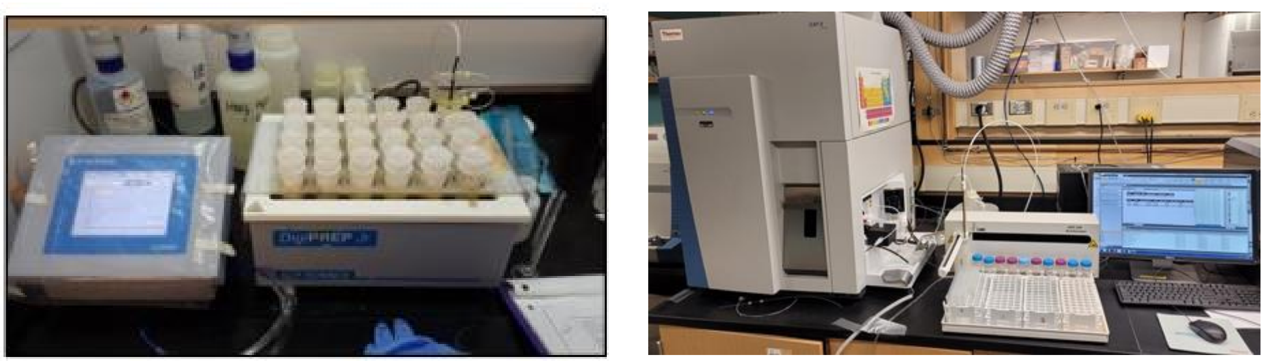


Figure 3.1: Nutritional quality analysis of crops grown in rock dust-based growth media amendment analysis. Flow diagram depicting processing procedures from crop harvesting in the growth chamber, drying, grinding, and biochemical analysis of the dried samples.

3.2.11 Mineral nutrition analysis (Acid extraction)

A 100 mg powered dry leaf sample was digested in 10 mL of trace nitric acid (70% v/v) using a Multiwave Go-microwave digestion system (Anton Paar GmbH, Graz, Austria). The powdered samples were weighed in sample vessels before digestion, and nitric acid was added prior to loading on the digester (Fig.3.2A). The microwave digester was operated under the following conditions: temperature ramped to 180 °C for 10 min followed by a constant temperature of 180°C for 20 min. After digestion, the samples were cooled and then diluted using 3% nitric acid as a solvent for analysis. A 43-standards calibration curve (ranging from 1 to 500 μL^{-1}) was prepared using IV-ICPMS-71A standard mixture (Inorganic Ventures, Inc; Christiansburg, VA 24073, USA). Aliquots of the digests were diluted with 3% nitric acid and spiked with rhodium-103 (final volume = 10 µL) as the internal standard for ICP-MS analysis. The ICP-MS analysis was conducted using the following parameters: an auxiliary gas flow of 0.79 L/min, nebulizer gas flow of 1.01 L min^{-1}, plasma gas flow of 14 L/ min^{-1}, RF power of 1548 W, detector mode, and a dwell time of 0.01 s using argon gas at a purity of 99.99%. Values are expressed as part per billion (ppb) (Fig.3.2B)



A **B**

Figure 3.2: Analytical processes A) Acid digestion of plant samples, B) ICP-MS nutrient analysis.

***3.2.*12 Statistical analyses**

Two growth cycles were completed for each crop grown in the growth chamber. Considering similar result patterns, the data were pooled for each crop and analyzed by one-way analysis of variance (ANOVA) using the XLSTAT Premium program (Addinsoft, New York, USA). There was a total of eight replication for each parameter considering two crop cycles. Where the treatments expressed significant effects, the differences between means were separated by Fisher's Least significant difference (LSD; $\alpha = 0.05$). We conducted multivariate analysis such as principal component analysis (PCA) to visualize the treatment groups to better understand how the media quality influences the crop agronomic performance and nutritional composition. Pearson's correlation analysis was performed to test the relationships between the media quality parameter clustering in the same quadrants with crop agronomic performance and nutritional composition. All figures were prepared using XLSTAT Premium version (Addinsoft Inc 244 Fifth Avenue, Suite E100 New York, N.Y. 10001) and SigmaPlot 13.0 software programs (Systat Software Inc., San Jose, CA).

3.3 Results and Discussion

This session is arranged and discussed by crop type and its agronomic performance as part one followed by the discussion of the antioxidant and phenolic properties also by crop type. The protein content discussion is a combination of all the three.

3.3.1 Effect of media amendment on agronomic performance of Kale

The chlorophyll content, plant height, root-shoot ratio, dry weight, number of leaves, and total biomass, were measured in different RD-based amendments as an indicator of the agronomic performance of kale. The plant chlorophyll content is a vital indicator of plant health and crop agronomic performance(Rorie *et al.*, 2011). The chlorophyll contents can give insights into the growth and productivity of plants through their role in photosynthesis (Wang and Grimm, 2021). Study results showed that different growing media amendments significantly affected the plant chlorophyll content in kale (Fig**.** 3.3A). Kale plants expressed increased chlorophyll contents when grown in the media amendment and other treatments compared with Promix as shown in Figure 3.3A. The lowest chlorophyll contents were recorded in kale plants grown in TS, having only topsoil (Fig. 3.3A). Crops cultivated in RD-based increased leaf chlorophyll contents in all treatments where it was used as natural growth media amendment. The treatment RD expressed 15% more chlorophyll contents than TS (Fig 3.3A). The observed increase in leaf chlorophyll content in the present study could be ascribed to the nutrient-rich combination of RD with other growth media, including biochar and Promix (Fig. 3.3A). For instance, the promix contains a high amount of N and P required for normal plant development and chlorophyll. Biochar has a high amount of C with a large surface area enabling the release of macro-and micronutrients in RD for the formation of high chlorophyll content(Nett *et al.*, 2012; McCormack *et al.*, 2013). Biochar has a high surface area, enabling it to have high porosity, high cation exchange capacity (CEC), and a high concentration of negative charge on its surface. These properties allow biochar to have good water retention and nutrient release for plant growth (Sashidhar *et al.*, 2020).

RD-based amendment (RCP) had significant effects on the height of kale. RCP treatment produced significantly higher plant height than the lowest in topsoil, huplaso, and other treatments. However, there was no significant difference between RCP and the control P, even though RCP showed a moderate growth over the control P (**Fig**. 3.3D). The above performance of rock dust-based growth media could result from the alkaline pH and cation exchange capacity of RD responsible for nutrient exchange to enhance crop growth (Plata *et al.*, 2021). Our results are consistent with the experiment conducted by Fyfe *et al.* (2006), who observed a significant increase in plant height and yield of watermelon, rice, cassava, and maize crops with RD application compared with the same crops fertilized with nitrogen(N), phosphorus(P_S) and potassium(K).

Our results contradict the experiment conducted by Jones *et al.* (2009). They found that adding fine-grained basaltic materials to compost and peat resulted in a significant 2-fold reduction in wheat biomass production and an overall reduction in most plant growth indicators in tomato plants grown in a pot experiment and harvested 2.5 months later. We observed a significant increase in the dry weight of crops grown in RCP and RBP compared with TS. This could be ascribed to the possible increase in C assimilation and partitioning, resulting in higher dry leaf matter content(Iheshiulo *et al.*, 2017). However, the crop produced in RBP and RCP showed a moderate increase in the dry weight when compared to the control P, but this was not statistically different. (Fig 3.3A). Similarly, RD-based amendments (RP, RCP, RBP and RP) affected the number of leaves produced by kale plants. In this treatment, the number of leaves produced was significantly higher compared to the universal soil and other treatments except for the control P, which showed no significant difference with the RD-based treatments (RP, RCP, and RBP).

We report here that growing media quality is inevitable in improving the growth and yield of crops. We demonstrated that the improved media amendments and their nutritional composition can alter plant health through the root-shoot ratio. For example, the root-shoot ratio of crops grown in RD amendment (RT)showed a significant increase over other amendments. RT showed no significant difference compared to the control P, RDT, and RBP (Fig.3.3E). The improvement of the crop agronomic performance by RD-based amendment could be ascribed to the improvement of RD in the mixture of other media in enhancing nutrients release for crop growth de Souza *et al.* (2013).

The present study agrees with similar research conducted by (Burghelea *et al.*, 2015), who revealed a significant increase in the root of *Stenotaphrum secundatum* (buffalo grass) grown in all three types of rocks (Basalt, schist, rhyolite, and granite) used in their experiment. However, the authors observed low biomass, which agrees with the present study when kale was grown in 100% RD (Fig.3.3F). Furthermore, a similar experiment conducted by Bolland and Baker (2000) revealed an increase in plant roots. However, it showed a reduction in the growth and yield of crops grown in RD-based amendment. Our results, however, contradict the previous finding by Campbell (2009), which revealed no effect on plant growth when field and pot experiments were conducted with RD and compost to evaluate crop performance. This contradiction could be explained because of the different parent material of RD, application rate, and plant species used in the referenced study.

The improvement in plant growth by a mixture of RD and other media (compost, biochar, and promix) could be ascribed to good water retention and nutrient supply by RD-based growing media. For instance, the low bulk density and high porosity observed in the RD-based amendments permitted the root development of the crops, available water capacity, and availability of nutrients for microbe activity, all of which have an impact on critical soil processes and crop production(Barbosa *et al.*, 2018; Indoria *et al.*, 2020). Water content is of prime importance for photosynthesis and biomass because their deficiency restricts nutrient uptake in plants and transpiration, which could induce stomatal closure affecting photosynthesis efficiency (Buckley, 2019). Additionally, due to the larger number of charged bonding sites on the peat (Promix) particle, peat has a higher CEC than mineral soil and so has a greater potential to store nutrients (Maher *et al.*, 2008). Kale crop grown in RD-based amendment in the growth chamber (Fig.3.4).

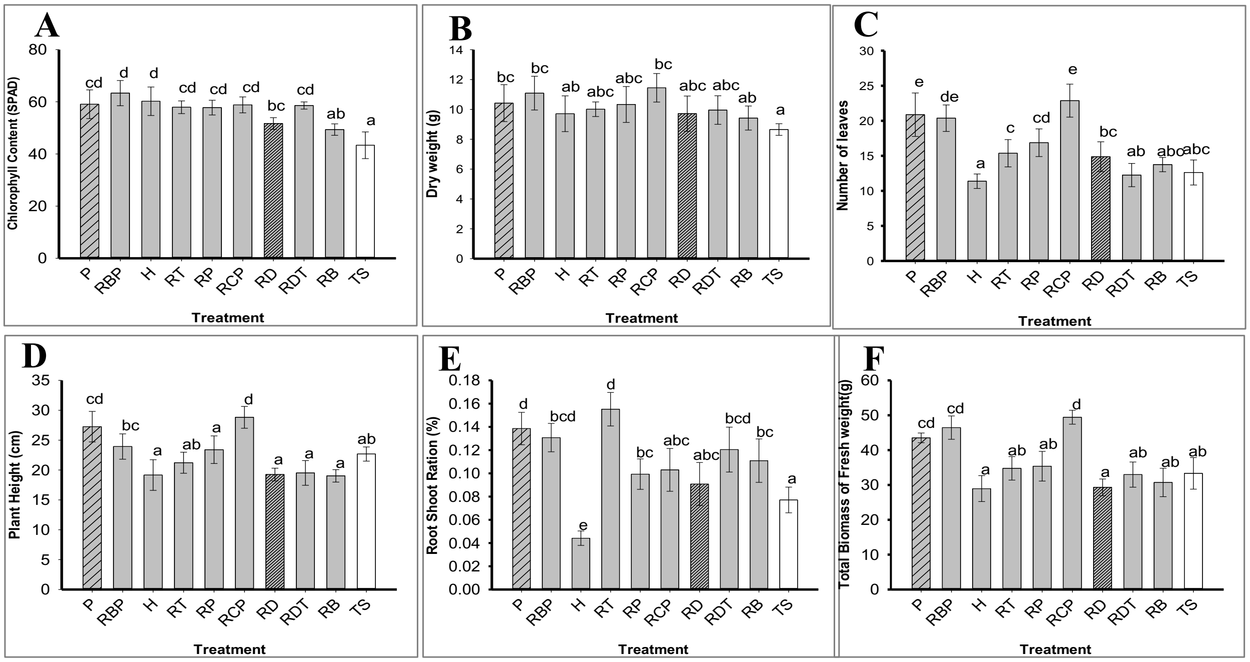


Figure 3.3. Effects of different growth media amendments on the agronomic performance of kale grown growth chamber: Chlorophyll content (A); dry weight (B); number of leaves (C); plant height (D); root shoot ratio (E) and Total biomass (F) in kale plants. P = 100%Promix(positive control), RBP = 50%Rock dust + 25%Biochar + 25%Promix. H = Huplaso(negative control), RT = 25%Rock dust + 75%Topsoil, RP = 50%Rock dust + 50%Promix, RCP =50% Rock dust +25% Compost + 25%Promix, RD =100% Rock dust(by-product), RDT = 50%Rock dust+50%Topsoil , RB = 50%Rock dust + 50%Biochar, TS = 100%Topsoil.

Figure 3.4: Kale plant (***Brassica oleracea***) grown in rock dust-based amendment (rock dust plus promix).

3.3.2 Effect of RD-based media amendment on agronomic performance of Lettuce

The following parameters, chlorophyll content, root-shoot ratio, plant height, dry weight, number of leaves, and total biomass, were used to assess the crop agronomic performance of lettuce. Our results showed significantly higher chlorophyll content in lettuce grown in P, RBP, RB, and RCP compared to when cultivated in100% RD (Fig. 3.5A), which had the lowest chlorophyll content. However, RB and RCP were not substantially different from the control (P) and TS. Our observation of the lowest chlorophyll content in crops grown in RD agree with previous research conducted by (Li and Dong, 2013), who observed a decrease in chlorophyll content of tomato plants when they evaluated the effect of RD used as fertilizer in maintaining soil health and tomato plant under greenhouse conditions. Their experiment was made up of four treatments, including

M (commercial fertilizer), A (RD soil amendment), M+A (commercial organic fertilizer + RD soil amendment), and CK (blank control). Additionally, they observed no significant effect of treatment A and M+A on plant height, stem diameter, and biomass. This variation could be because other treatments could have different particle sizes for water retention, variation in nutrient release patterns (Ro*y et* al.,2010; Sisouvan*h et* al., 2010).

The root-shoot ratio increased surprisingly in crops grown in 100%RD in our study. Our result agrees with previous research, which revealed that RD influenced crop roots (dos Santos Sousa *et al.*, 2021). However, the increase in root-shoot ratio did not translate into a significant increase in the total biomass of crops grown in 100% RD. These results also agree with the experiment of (Bolland and Baker, 2000), who revealed a reduction in total biomass of crops grown in RD. We, however, observed that when 50% of RD was mixed with 50% of Promix, the total biomass increased. A similar trend was observed when 50% RD was added to 25% biochar and 25% Promix (Fig.3.5F).

Plant heights were measured at the crop's maturity, and the results are presented in (Figure 3.5D). Medium RCP showed better performance compared to other treatments. RCP showed a significant increase in plant height over TS and other treatments but was not significantly different from RP and the control P. The increased plant growth could be attributed to the medium's improved soil moisture availability, and appropriate pH. Our results agree with a previous experiment (Pereira *et al.*, 2020).The purpose of their study was to see how different rates of bovine manure and rock powder (RP) affected growth characteristics, chlorophyll concentration, physiological indices, and the production of (*B. oleracea L. var. acephala*). Their media amendment was made up of bovine manure (60, 120, 180, and 240 g pit^{-1}) combined with doses of rock powder (6, 12, 18, and 24 g pit^{-1}). The result of their experiment revealed an increase in crop growth when 180g of bovine manure and 18g RD increased plant height, diameter, and the number of the leaf.

The present experiment observed the effect of media RCP, RP, and RT on the dry weight of lettuce. Media RCP and RP showed a significant increase in dry weight over TS and other treatments but were not significantly different from the control P. The lowest dry weight was recorded in RD. Similarly, media RCP, RP, and RBP showed a significant increase in the number of leaves and total lettuce biomass but were not significantly different from the control P. The variations in the effect of RD-based amendments on plant growth and yield are most likely due to differences in soil characteristics and the media amendment mineral content. Our results concur with the previous experiment conducted by Mohammed *et al.* (2014) who used K from feldspar, mica, and nepheline + microcline to test plants cultivated in sandy soils, with leek (*Allium ampeloprasum var. porrum*) Natural and artificial soil was used in the pot studies, which were conducted under controlled environmental conditions. Growth rates with nepheline syenite (natural soil) and microcline (both soils) did not differ significantly from the negative control, but leek shoot K content was significantly higher with nepheline syenite, demonstrating the availability of K from this source. However, the present study contradicts a previous study conducted by Jones *et al.* (2009). They observed a reduction in leaf fresh and dry weight (59% reduction) when RD application was used to grow tomatoes compared to waste-derived compost without RD. Lettuce crop grown in RD-based media compared with control (Fig.3.6).

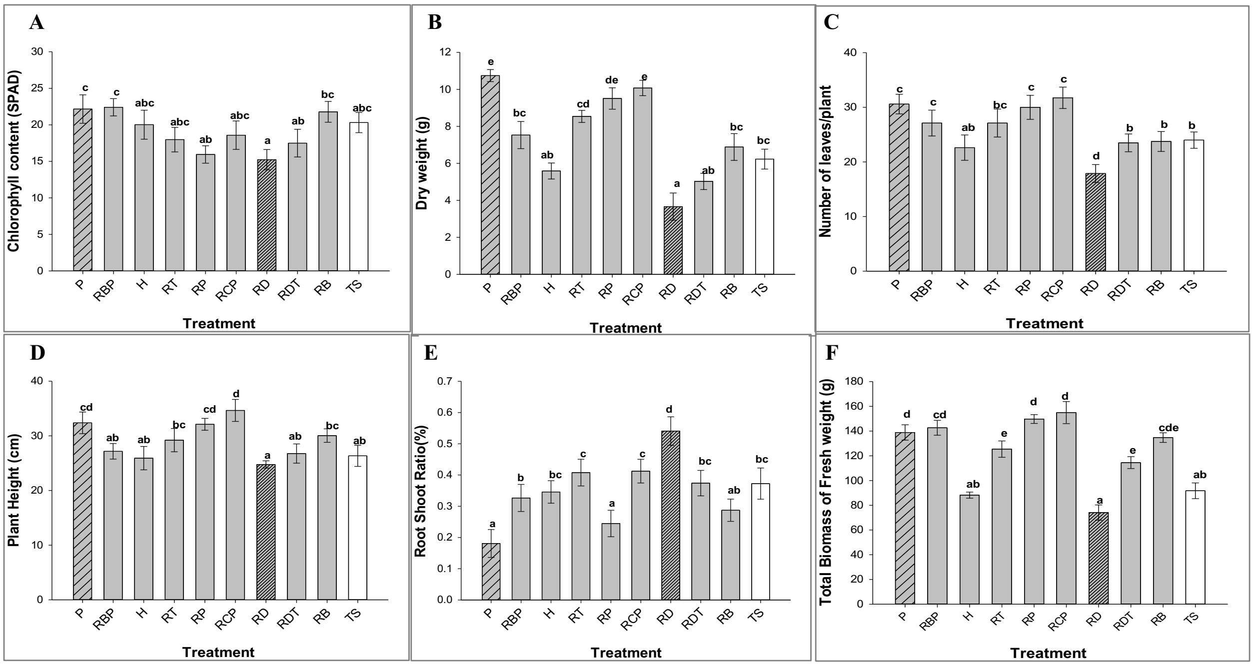


Figure 3.5 Lettuce crop grown in rock dust and rock dust-based amendments in growth chamber. The agronomic performance of lettuce in 10 treatments are displayed for (A) chlorophyll content, (B) dry weight, (C) number of leaves, (D) plant height (E) root shoot ratio and (F) total biomass of fresh weight. P = 100%Promix(positive control), RBP = 50%Rock dust + 25%Biochar + 25%Promix. H = Huplaso(negative control), RT = 25%Rock dust + 75%Topsoil, RP = 50%Rock dust + 50%Promix, RCP =50% Rock dust +25% Compost + 25%Promix, RD =100% Rock dust(by-product), RDT = 50%Rock dust+50%Topsoil , , RB = 50%Rock dust + 50%Biochar, TS = 100%Topsoil.

Figure 3.6: Lettuce (*Lactuca sativa*) crops grown in various rock dust-based amendments compared with promix media and 100% rock dust media.

3.3.3 Effect of RD-based media amendment on agronomic performance of Amaranth production in controlled environmental conditions.

The chlorophyll content, plant height, root-shoot ratio, dry weight, number of leaves, and total biomass, were measured in different RD-based amendments as an indicator of the agronomic performance of amaranth. Chlorophyll is essential for photosynthesis in plants and an essential mediator of the plant growth response under different cultivation or management conditions.

Our result demonstrated higher chlorophyll content of amaranth grown in Promix (control), which was statistically at par with crops cultivated in RB and RBP treatments. The lowest chlorophyll level was noted for crops cultivated in TS (Fig. 3.7A). The increase in chlorophyll content of amaranth cultivated in RB, RBP, and Promix over TS and other treatments could be ascribed to various factors. First of all, the low bulk densities (data not shown) of RB, RBP and Promix potting media indicate an excellent water retention ability to dissolve mineral salts and transport nutrients for plant uptake(Li *et al.*, 2018). Furthermore, plants adjust to different media amendment/environments to maximize photosynthesis depending on the element(e.g., N and P_s) required for chlorophyll increase(Li *et al.*, 2018). Consistent with the elevated chlorophyll content, we noted significant increases in dry weight of amaranth cultivated in RB compared to crops grown in TS. This increase was similar to plants cultivated in Promix (P) used as the control. (Fig 3.7B). Furthermore, amaranth produced in RP and RB media showed a significant (P =0.05) increase in the number of leaves compared to all other treatments except in the control (P) where the performance was similar (Fig. 3.7C).

The improvement in crop performance could be ascribed to the combination of Promix and biochar with RD, which might have contributed to the observed crop improvement due to their physicochemical properties. Biochar has been reported to contain high carbon content combined with its surface area, organic matter content, and highly porous nature, which may create a conducive environment to support microorganisms growth and their metabolism of nutrients in the substrate known to be important mediators of plant growth and development (de Amaral Leite *et al.*, 2020).

Our observation of an increase in plant height, chlorophyll content, and total biomass of crops grown in RD amendment agrees with a previous experiment conducted by Basak *et al.* (2018). We observed an increase in the root-shoot ratio of amaranth produced in 100% RD and RP compared with crops cultivated in control (P) and all the other treatments (Fig. 3.7E). Our result agrees with dos Santos Sousa *et al.* (2021); their goal was to see how different effective microorganisms (EM) doses affected the growth of the iceberg lettuce cultivar and Lucy Brown when administered with or without RD. The authors obtained results similar to the present study when RD was mixed with substrate EM, with a significant effect on root length, leaf area, and shoot fresh matter of lettuce. The growth performance of amaranth crop grown in the growth chamber (Fig.3.8)

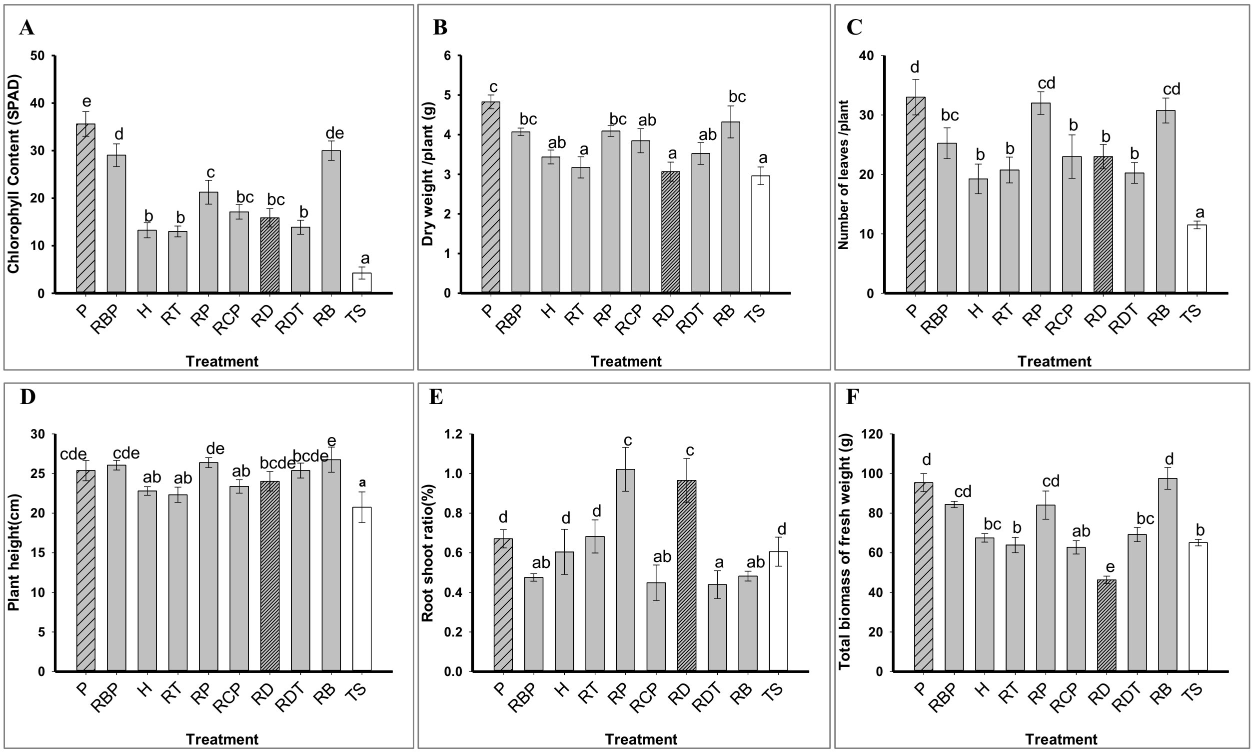


Figure 3.7 Amaranth crop grown in rock dust and rock dust-based amendments in growth chamber. The agronomic performance of Amaranth in 10 treatments are displayed for (A) chlorophyll content, (B) dry weight, (C) number of leaves, (D) plant height (E) root shoot ratio and (F) total biomass of fresh weight. P = 100%Promix (positive control), RBP = 50%Rock dust + 25%Biochar + 25%Promix. H = Huplaso (negative control), RT = 25%Rock dust + 75%Topsoil, RP = 50%Rock dust + 50%Promix, RCP =50% Rock dust +25% Compost + 25%Promix, RD =100% Rock dust(by-product), RDT = 50%Rock dust+50%Topsoil, RB = 50%Rock dust + 50%Biochar, TS = 100%Topsoil,

Figure 3.8: Amaranth (*Amaranthus Viridis*) grown in rock dust-based amendments

3.3.4 Assessment of the nutritional quality of Kale produced in RD-based media.

The current study focus, and consumer interests are geared toward increasing cultivated crop yield and nutrient content through sustainable practices. We next sought to assess kale's phenolic and antioxidant content to understand better how RD-based media may improve the nutritional quality of the crop produced. For years, the health and nutrition industries have paid attention to kale due to its exceptional nutritional profile. Kale contains antioxidants, carotenoids, glucosinolates, lipid-soluble tocopherols, ascorbic acid, mineral nutrients, and dietary polyphenols, among other important health-promoting compounds. These substances play critical roles in human health and disease prevention(Zhao *et al.*, 2007; Hahn *et al.*, 2016; Zhang *et al.*, 2017; Abbey *et al.*, 2018b).

Our results showed low levels of phenolic content in kale grown in RD-based amendments.

Surprisingly, we observed that the RD-based amendments performed better or equally as good as crops cultivated in the three controls evaluated in this study (P, TS, and H). Promix is recognized as a high-quality potting medium for commercial vegetable production.

Our results showed that all the RD-based amendments had an effect on the antioxidant capacity of kale when analyzed by FRAP and ABTS methods. The control P had the highest antioxidant content of kale but was not significantly different from RBP. Medium RD recorded the lowest antioxidant content of kale (Table 3.2). The lowest antioxidant content of RD may be due to an insufficient amount of nitrogen in RD. RD-based media produced kale crops with similar or superior antioxidant and phenolics content compared to the crops cultivated in the negative control media H and TS (universal soil) (Table 3.2), indicating the potential application of RD as a media amendment to produce kale with enhanced antioxidants. Effects of the RD-based media amendments on the phenolic and antioxidant content could be ascribed to the improved media quality influenced by the ability of RD to release nutrients, improve soil pH, and water retention, etc. (Forján *et al.*, 2016; Basak *et al.*, 2021). Our findings show that kale produced in RD-based amendment can provide a significant quantity of antioxidants.

Table 3.2. Total phenolic content (TPC) and total antioxidant activity (TAA) of Kale crop grown in different mixed growing media

Treatment	TPC (mg QE/g DW)	TAA FRAP (µmol Trolox/g DW)	TAA ABTS (µmol /g sample)
P	0.55 ± 0.05^{bcd}	452.38 ± 15.20^{c}	227.74 ± 3.27^{ab}
RBP	0.49 ± 0.06^{abc}	445.03 ± 4.31^{c}	292.93 ± 6.81^{de}
H	0.60 ± 0.08^{d}	339.79 ± 16.30^{ab}	257.10 ± 4.29^{c}
RT	0.65 ± 0.06^{cd}	333.84 ± 14.88^{ab}	329.67 ± 4.60^{f}
RP	0.25 ± 0.01^{a}	349.55 ± 9.49^{ab}	254.81 ± 8.82^{bc}
RCP	0.42 ± 0.02^{ab}	396.83 ± 12.36^{bc}	286.32± 4.92^{d}
RD	0.53 ± 0.12^{cd}	292.47 ± 11.85^{a}	248.89 ± 3.25^{bc}
RDT	0.28 ± 0.02^{a}	361.21 ± 14.67^{ab}	201.87± 8.32^{a}
RB	0.53 ± 0.08^{cd}	373.86 ± 17.05^{b}	319.74 ± 4.73^{ef}
TS	0.38 ± 0.08^{ab}	389.71 ± 17.30^{bc}	255.66 ± 7.54^{bc}

RD = Rock dust, TS = Topsoil, H = Huplaso, P = 100%Promix(positive control), RBP = 50%Rock dust + 25%Biochar + 25%Promix. H = Huplaso(negative control), RT = 25%Rock dust + 75%Topsoil, RP = 50%Rock dust + 50%Promix, RCP =50% Rock dust +25% Compost + 25%Promix, RD =100% Rock dust(by-product), RDT = 50%Rock dust+50%Topsoil , RB = 50%Rock dust + 50%Biochar, TS = 100%Topsoil.Values represent means ± standard errors; n = 4 and different letters show significant differences between treatments at α = 0.05.

3.3.5 Effect of media amendment on the antioxidant and phenolic content of lettuce grown in different media amendments.

Fruits and vegetables have numerous health benefits to humans, including reducing various diseases, including cardiovascular disorders, possibly because of the antioxidant properties of these vegetables and fruit (Liu, 2013; Zhang *et al.*, 2015).

We observed that the hydrophilic (HAA) and lipophilic (LAA) phenolic content of lettuce leaves were significantly influenced by the RD-based amendments (data not shown). Our results showed a significant increase in the total phenolic (TPC) content of crops grown in RB, RT, and RD compared to crops grown in control (P) and the remaining amendments (Table 3.3). Although the Lettuce crops grown in 100% RD showed a significant increase in total phenolic content compared to the control (P), this increase was not significant compared with crops cultivated in topsoil (Table 3.3). Our results are consistent with an experiment conducted by Abbey *et al.* (2018a), who observed improvement in antioxidant and phenolic content of kale cultivated in volcanic minerals and k-humate based media amendment under control environmental conditions.

The antioxidant capacity of leafy vegetables is essential because of their ability to prevent oxidative damage in both plants and humans (Lobo *et al.*, 2010). Several epidemiological studies have found links between a high-vegetable diet and lower rates of severe diseases. There is evidence that eating a lot of greens, such as amaranth, can help prevent devastating diseases, including cancer, diabetes, stroke, and cardiovascular disease. It is believed that the presence of phenolics and vital minerals, vitamins, omega-3 fatty acids, and antioxidants, reduces the likelihood of developing disease. These phytochemicals are responsible for determining the nutrient level or quality of the crop and the perceived health advantages to the end-user or consumer (Abbey *et al.*, 2018a)

Our study observed a significantly higher ($p<0.05$) total antioxidant activity (TAA) in the crop grown in the following amendments: RD, RB, RBP, RCP, RT, and H compared to the control (P) as demonstrated by both the ABTS and FRAP assays (Table 3.3**).** The nutritional quality could be attributed to the improved agronomic performance linked to RD's ability to improve growing media's physical and chemical properties. Additionally, the presence of a high amount of nitrogen in Promix used in the RD-based amendment formulation could also be a factor contributing to the improved nutritional quality. Lastly, pH and other mineral nutrients can alter the uptake and assimilation of mineral nutrients, influencing the synthesis of secondary metabolites in lettuce plants.

Table 3.3: Total phenolic content (TPC) and total antioxidant activity (TAA) of Lettuce crops grown in different media amendments.

Treatment	TPC (mg QE/g DW)	TAA FRAP (µmol Trolox/g DW)	TAA ABTS (µmol /g sample)
P	33.26 ± 0.27^{b}	145.21 ± 1.51^{abc}	247.96 ± 7.68^{a}
RBP	30.50 ± 0.07^{a}	150.15 ± 1.73^{abc}	329.98 ± 7.03^{cd}
H	29.93 ± 0.18^{a}	58 ± 19.85^{a}	301.10 ± 15.66^{b}
RT	38.51 ± 0.61^{d}	191.10 ± 2.01^{d}	461.31 ± 8.10^{e}
RP	30.19 ± 0.20^{a}	168.65 ± 5.73^{cd}	304.22 ± 5.85^{bc}
RCP	29.46 ± 0.80^{a}	165.63 ± 1.09^{bcd}	336.16 ± 8.09^{d}
RD	36.00 ± 0.45^{c}	180.01 ± 1.96^{cd}	241.35 ± 5.43^{a}
RDT	33.39 ± 0.88^{b}	127.56 ± 1.47^{ab}	324.88 ± 6.88^{bcd}
RB	42.00 ± 0.76^{e}	190.17 ± 2.34^{d}	323.19 ± 8.21^{bcd}
TS	37.19 ± 0.51^{cd}	181.77 ± 2.32^{cd}	244.51 ± 12.49^{a}

. P = Promix(positive control), RBP = 50%Rock dust + 25%Biochar + 25%Promix. H = Huplaso(negative control), RT = 25%Rock dust + 75%Topsoil, RP = 50%Rock dust + 50%Promix, RCP =50% Rock dust +25% Compost + 25%Promix, RD =100% Rock dust(by-product), RDT = 50%Rock dust+50%Topsoil , RB = 50%Rock dust + 50%Biochar, TS = 100%Topsoil

. Values represent means ± standard errors, n = 4 and different letters show significant differences between treatments at α = 0.05.

3.3.6 Antioxidant and phenolic content of amaranth grown in different media amendment*s.*

Amaranth is a gluten-free plant with excellent nutrient content and antioxidant in its leaves. Amaranth crop has been reported to have high antioxidant content (Karamać *et al.*, 2019; Sarker *et al.*, 2020). Analysis of the antioxidant content of amaranth leaves with the FRAP method showed no significant difference among RBP, H, R.P., RDT, T.S. treatments compared with crops cultivated in the control P (Table 3.4). A similar trend was observed in the ABTS method with an increase in total antioxidant, where amaranth cultivated in RBP had the highest antioxidant content. Consistent with our observation of the antioxidants in lettuce and kale cultivated in rock dust-based media amendments (Table 4), levels were similar or better than crops cultivated in the topsoil, huplaso, and promix as control treatments. These findings support the results of (Linares, 2019) who reported an increase in antioxidants of vegetables produced in RD-based media amended with worm casting compared with synthetic fertilizer. Furthermore, we observed that the phenolic content of crops grown in RDT increased substantially over that of crops cultivated in control (P). Still, this increase was not significantly different from crops cultivated in H and TS (Table 4), which are commercial products already on the market.

To the best of our knowledge, there is little information on the antioxidant of crops produced in RD-based amendments. The improvement of antioxidant in some RD-based amendments (RBP, RP, RDT, and RD) which performed better or equal to the control P in the present study indicates that RD mixed with other organic media could provide a suitable growth substrate capable of enhancing vegetable crop growth and nutritional quality.

Table 3.4: Total phenolic content (TPC) and total antioxidant activity (TAA) of amaranth crop grown in different media amendments**.**

Treatment	TPC (mg QE/g DW)	TAA FRAP (µmol Trolox/g DW)	TAA ABTS (µmol /g sample)
P	0.27 ± 0.03^{a}	443.84 ± 2.76^{cd}	547.26 ± 28.60^{ab}
RBP	0.30 ± 0.06^{ab}	455.61 ± 9.06^{d}	680.96 ± 31.19^{b}
H	0.39 ± 0.02^{abc}	413.43 ± 14.71^{abcd}	587.28 ± 31.09^{ab}
RT	0.51 ± 0.04^{abcd}	377.06 ± 10.98^{ab}	438.73 ± 23.13^{a}
RP	0.35 ± 0.04^{ab}	449.04 ± 17.96^{cd}	430.26 ± 23.44^{ab}
RCP	0.36 ± 0.08^{ab}	384.16 ± 4.70^{ab}	346.48 ± 27.97^{a}
R.D.	0.48 ± 0.07^{abc}	389.24 ± 11.40^{abc}	616.69 ± 11.22^{b}
RDT	0.61 ± 0.04^{cd}	427.86 ± 8.75^{bcd}	631.29 ± 13.32^{b}
RB	0.52 ± 0.15^{bcd}	358.32 ± 4.80^{a}	470.33 ± 27.67^{a}
TS	0.73 ± 0.16^{d}	409.41 ± 9.35^{abcd}	582.51 ± 20.94^{ab}

P = 100%Promix(positive control), RBP = 50%Rock dust + 25%Biochar + 25%Promix. H = Huplaso(negative control), RT = 25%Rock dust + 75%Topsoil, RP = 50%Rock dust + 50%Promix, RCP =50% Rock dust +25% Compost + 25%Promix, RD =100% Rock dust(by-product), RDT = 50%Rock dust+50%Topsoil , RB = 50%Rock dust + 50%Biochar, TS = 100%Topsoil. Values represent means ± standard errors; n = 4 and different letters show significant differences between treatments at α = 0.05.

3.3.7 Protein content of amaranth, lettuce, and kale grown in rock dust-based media amendments.

This study measured and compared the protein content of three horticultural crops produced in RD-based amendments. In many research studies, plant proteins have been shown to reduce cardiovascular and metabolic risk factors(Hertzler *et al.*, 2020). To the best of our knowledge, there is limited literature on the protein content of amaranth crops produced in RD-based amendments. The results obtained in this study showed no significant difference in the protein content in both kale and lettuce crops grown in RD-based amendments compared to the control

(Fig 3.9 A and B). However, the media amendment had a significant effect on the protein content of the amaranth crop. Medium RCP showed a significant increase with the highest protein content over all the other amendments (Fig.3.9C). The control P recorded the lowest protein content (Fig 3.9C). The improvement could be ascribed to the mixture of RD, compost, and promix, which leads to the addition of organic matter to the media to enhance both the physical and chemical qualities of the media, as well as providing, nitrogen and other vital nutrients needed for protein production(Forján *et al.*, 2016).

Additionally, the improvement of microorganisms (e.g., G+, G-,Protozoa, and Fungi) in the media may have facilitated the conversion of unavailable nutrients (e.g., P, and N) to available forms, providing plants with essential elements needed for protein formation (Agamy *et al.*, 2013). For instance, the plant takes nitrogen from the media made by bacteria. The bacterial convert nitrogen into amino acids and amino acids into protein. The value of amaranth is considered as a plant-based superfood, and its cultivation with RD-based media amendments can significantly enhance the protein content and nutritional quality of amaranth as a new food crop or an improved source of plant-based protein.

Previous researchers have revealed that the seeds and leaves of amaranth are more nutritious than those of other cereals. The protein level of amaranth grain is 15% higher than that of other cereal grains, and the lysine content is three times higher than that of maize(Bressani, 1989). edible amaranth stems and leaves are low-cost vegetables that contain a lot of protein with important amino acids like methionine and lysine, dietary fiber, carotenoids, vitamin C, and minerals like calcium, magnesium, potassium, phosphorus, iron, zinc, copper, and manganese(Sarker *et al.*, 2014; Chakrabarty *et al.*, 2018). Amaranth has been shown to positively impact the lipid spectrum even when it is devoid of fat. This finding has deduced that the protein fraction or other components may be the reason for this phenomenon(Barral and Llois, 2002; Berger *et al.*, 2003; Mendonça *et al.*, 2009).

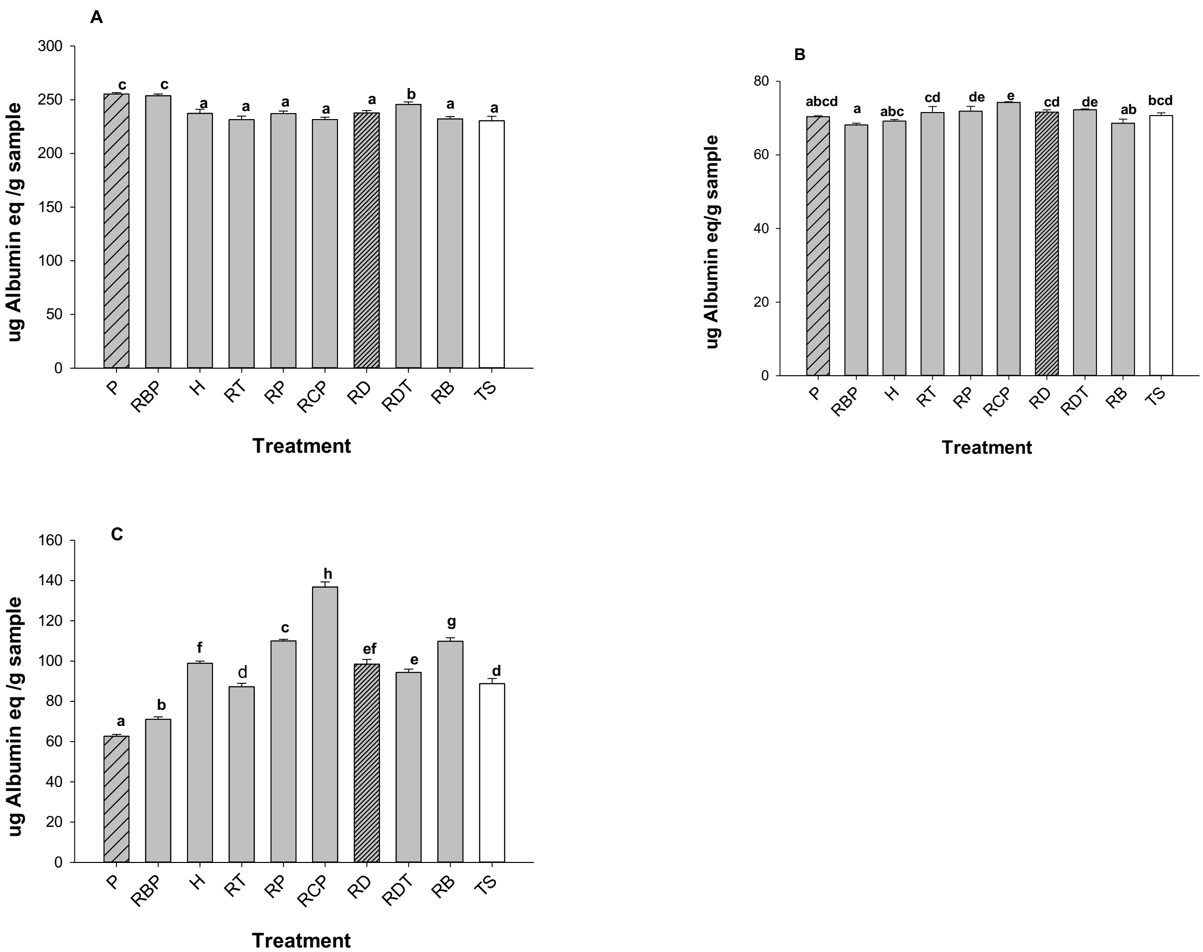


Figure 3.9: Protein content of kale, amaranth, and lettuce grown in rock dust-based amendments A=protein content of kale, B=protein content of lettuce and C=protein content of amaranth plant. The values in bar chart represent means ± standard errors; n = 4 and different letters show significant differences between treatments at α = 0.05

3.3.8 Sensory Evaluation of Amaranth and Lettuce crop grown in rock dust-based amendment

Amaranth was cultivated for the first time during this study as a new vegetable crop in Newfoundland and Labrador. We conducted a sensory analysis to gauge consumer preference and acceptability of amaranth as a new vegetable crop and determine the following parameters: the taste, colour, flavor, overall liking, the overall appearance of steamed amaranth whether these parameters would influence their choice or perception. The results from the sensory analysis of the cooked (steamed) amaranth crop revealed consumers gave the highest ratings based on the color of the leave (Fig.3.10A). Colour is an important parameter that can significantly contribute to acceptability and consumer choice preference (Francis, 1995). The flavor, overall preference, and purchasing preference were among the highest-rated parameters, confirming the consumer's acceptance of amaranth as well as liking and willingness to buy an amaranth crop produced from RD-based soil amendments.

Similar to Amaranth, lettuce produced in RB had the highest consumer preference and overall preference (Fig.3.10 B). Consumers preferred crops grown in RB because they liked their overall appearance, and this was driven primarily by the superior size of the crops cultivated in this media amendment (Fig.3.11A). This outcome may be due to the combination of these two substrates having an impact on nutrient retaining capacity, better element exchange, and optimal moisture and air distribution, all of which had an impact on root formation, nutrient absorption, and plant growth in general. (Sedaghat *et al.*, 2017). Participants in the food sensory lab under taking the sensory evaluation (Fig.3.11)

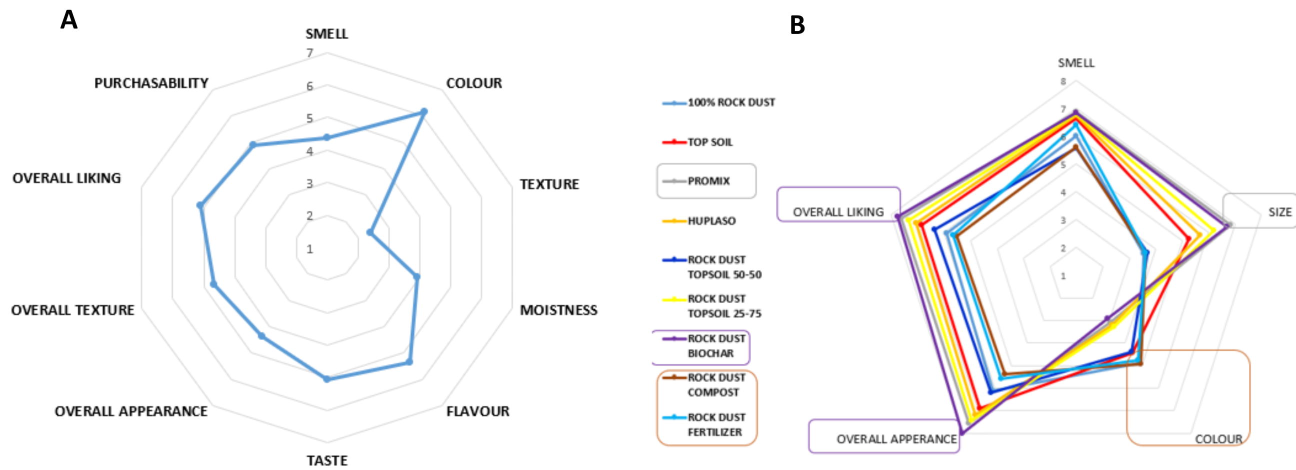


Figure 3 Sensory analysis of Amaranth and Lettuce. (A) The spider chart shows the sensory/perceptual attributes of cooked Amaranth stew, including smell, color, texture, moistness, flavor, taste, overall appearance, overall texture, overall preference, and purchasing preference. (B) The spider chart shows the sensory/perceptual attributes of lettuce plants' physical characteristics, including smell, size, color, overall appearance, and overall liking. The scale of preference was 1−8, indicating the lowest to the highest preference, respectively.

Figure3.11: Sensory evaluation A) Comparing Promix (control) with RB, B) Participant comparing crops and answering the evaluation questionnaire on lettuce plant c) Participants tasting steamed amaranth and evaluating its sensory characteristics D) Student evaluating amaranth plants grown in RD-based amendments at the functional food sensory lab at Grenfell campus.

3.3.9 Relationship between media quality and influences on agronomic performance and nutritional quality of vegetable crops grown in rock dust-based amendments in a controlled environment

The overall results obtained from this study showed that rock dust-based media amendments enhanced the agronomic performance and nutritional quality of vegetable crops cultivated under controlled environment conditions. We next performed a multivariate statistical approach to better understand how media quality contributes to the improved agronomic performance and nutritional quality.

Results from the PCA assessing the growth media quality and amaranth agronomic performance as well as nutritional quality explained about 60% of the total variability in the dataset, where PC1 displayed 37.1%, and PC2 displayed 22.8% of the total data variability (Fig. 3.12A and 3.12B). The observation plot depicted distinct segregation of the ten growth media amendments based on the centroids where RD and RBP amendments were grouped in Q-1 of the observation plot (Fig. 3.12A). Growing media H was separated in Q-2; RDT, RT, P, and TS were segregated in Q-3, whereas RB, RP, and RCP were clustered in Q4 (Fig. 3.12A and 3.12B). The biplot displays the relationship among different observed variables where bulk density, protein, photosynthesis, media pH, G^+ & G^- bacteria, fungi, arsenic (As), beryllium (Be), and boron (B) clustered with RBP media amendments as shown in (Fig. 3.12A and 3.12B). Huplaso separated in Q-2 with iron (Fe), TAA(FRAP), Toxic minerals, sodium (Na), aluminium (Al), iron (Fe), magnesium (Mg), Fungi(F), boron(B), and Sulphur(S) (Fig. 3.12A and 3.12B).

RDT, RT, Promix, and TS were clustered in Q3 with plant height (PLH), total biomass (TBM), and crop quality (TAA(ABTS)), mineral composition of the leaves (magnesium (Mg), manganese (Mn), iron (Fe), selenium (Se), Total macro minerals), and media quality (potassium(K), phosphorus (P_s), Protozoa (1P), copper (Cu), zinc (Zn), Porosity) (Figure 3.12A and 3.12B). Similar trends were observed when kale and lettuce were grown in the same media amendments (Fig. 3.12C and 3.12D) (kale) and (Fig. 3.12E and 3.12F) (lettuce).

To further improve our understanding of different parameters and their relationship with each other, we conducted Pearson's correlations to assess the strength and significance of the association between media parameters that segregated with the nutritional and agronomic performance in each media amendment (Table:3.5- 3.7). In kale production, we observed a strong significant negative correlation of media bulk density with G+&G-&F bacteria (r=-0.81) in Q-1 when kale was produced in RD and RBP (Table 3.5).

Additionally, strong significant positive correlations were also observed in amaranth production among the following parameters in Q3 (Table 3.7): (1) Gram positive bacteria (G+) (r=0.68) with zinc (Zn)(r=0.80), potassium(K)(r=0.74), phosphorus (P_s)(r=0.71), and plant total biomass(r=0.68); (2) gram positive bacteria (G+) with fresh weight (Fw) (r=0.79), (3). Porosity showed strong positive significant correlation with fresh weight(r=0.74) and total biomass(r=0.80) (Table 3.7).

The strong association of the microbial composition with the crop nutritional quality in different quadrants of the biplot suggests that the microbial population in the different media was instrumental in the mineralization and bioaccumulation of these elements as a nutrient in the crops cultivated in the respective rock dust based media (Lu *et al.*, 2013). Soil microbes excrete organic acids and carbon dioxide that react with water to generate carbonic acid to aid in the disaggregation and dissolution of minerals to influence the agronomic and nutritional quality of crops(Uroz *et al.*, 2009).

Furthermore, Li *et al.* (2021) reported additional mechanisms by the microbial community, including redox reaction catalysis and biological polymer hydrolysis. These increase water retention duration on the mineral surface and interact with ions by complexing and lowering their solution saturation state. One of the most important factors influencing biological activities is the availability of moisture by the medium in providing oxygen and nutrients(Lu *et al.*, 2013).

The gram-positive and gram-negative bacteria showed a strong positive significant correlation with chlorophyll content (CT)(r=0.59) when amaranth was grown in RBP, RCP, and RP media amendments (Table 3.7). In lettuce production, we observed in Q1 a strong negative significant correlation of pH(r=-0.89) with gram-positive, gram-negative bacteria and fungi(G+&G-&F). Bulk density showed a strong negative significant correlation with G+&G-&F(r=-0.81) when lettuce was grown in RBP and RD (Table 3.6).

The above correlation of the microbial community with the growing media in this study is evidence of how the media influence microbial community composition and nutrient cycling. For example, different pathways of microbes interact with plants and soil to enhance plant growth through nutrient transfer (e.g., nitrogen fixation, enzymatic decomposition of organic matter in the soil) and direct promotion of growth through phytohormones (Lyu *et al.*, 2021). Plant and microbes have a symbiotic relationship (Backer *et al.*, 2018). For example, plants can alter the soil's chemical and physical properties, like the development of roots to remodel the soil structure create more space for oxygen circulation, and release root exudates (López-Angulo *et al.*, 2020). Additionally, previous experimental studies showed that RD increase microbial activities through various factors, which includes the supply of trace elements, provision of microsites to the microbial community, and finally, the provision of buffering potential toxic elements (Bolland and Baker, 2000; Li and Dong, 2013; Manning, 2018).

The strong negative correlation observed between bulk density and microbial community in our current study may account for the improvement in the active microbial community leading to enhanced nutrient mobilization for crop growth. Our findings are consistent with the experiments conducted by Li *et al.* (2020), who evaluated compost fortified with rock dust to examine effects on apple orchard's soil properties, microbial activities, and yield. The authors observed a significant increase in microbial activities as well as an increase in yield.

However, our present study contradicts previous studies conducted by (Ramezanian *et al.*, 2013), who used volcanic rock dust as soil amendment compared with three-peat horticultural soils. The authors observed no effect on the media amendment and no yield effect on the agronomic performance of the wheat crop. Our study was conducted on vegetable crops under a controlled environment, whereas the comparative study was done on wheat under field conditions. This may account for the contrasting results.

In Q3, of (Table 3.6) we observed a strong positive significant correlation of protozoa (*1P*) with photosynthesis(r=0.57), dry weight (DW)(r=0.92), and mineral uptake of sodium (Na)(r=0.60). Porosity showed strong positive significant correlation with gram positive bacteria (r=0.75), protozoa(P)(r=0.69) and sodium uptake(r=0.62); potassium(K) showed strong positive significant correlation with photosynthesis(r=0.52). In Q4; we observed strong positive correlation of monosaturated fatty acid with FW(r=0.77),number of leaves(NL)(r=0.66), and Total biomass of fresh weight(TMBF),(r=0.83)(Table 3.6).Field capacity showed strong positive correlation with gram positive bacterial (r=0.59),cation exchange capacity showed strong positive correlation with number of leaves(r=0.66),(Table 3.6).Similar trend was observed in Kale crop grown in rock dust-based media amendments.

The release of nutrients from the media amendments for plant uptake directly relates to pH, which influences agronomic performance, crop quality, and mineral composition; soil pH enhances the soil enzyme activities and biodegradation of organic matter. For instance, pH controls mineralization in the growing media amendment through its immediate impact on microbial communities and their activities (Neina, 2019). The present research work revealed a strong significant positive correlation between K, crop agronomic performance, and plant height. Chlorophyll content and photosynthetic activities of the crops understudied showed a strong positive significant correlation with P, indicating the relationship between media quality, agronomic performance, and crop quality (Fig. 15).

Additionally, the relationship observed between microbial community and the total biomass of the current research was due to the presence of the gram-positive bacteria and eukaryotes present in the RD-based media amendments. Gram-positive bacteria showed a strong positive significant correlation with total biomass. This relationship could be associated with the strong cation exchange capacity(CEC) of RD, which is known to mediate the ability of RD to hold and release positive ions to create a conducive environment for prolific growth and survival of these microbial communities (Beerling *et al.*, 2018).

The application of RD in our study was from a volcanic source consisting of sand, silt, and clay. Amending RD with biochar (e.g., RBP), where both have properties of large surface area, makes them capable of holding plant nutrients loosely on their surface for release for plant root uptake. For instance, the RD particle size, made up of clay properties and minerals, could be responsible for the nutrient release through weathering by microbial activities. At the same time, the surface area and organic matter content of biochar could also be responsible for preventing the leaching of plant-available nutrients needed for crop growth (Saha *et al.*, 2020).

The surface area and organic matter content of media amendments and their ability to hold and release nutrient uptake for crop growth have previously been reported (Ramos *et al.*, 2017). Our analysis of the nutritional composition of the rock dust material used in the media formulations in this study showed a good proportion of cations like $Ca^{2+,}$ Mg^{2+}, K^{+}, and Na, relative to the proportion of cations like Al^{3+} and H^{+} (data not shown)(Arnott *et al.*, 2021). This can be ascribed to the mineral composition of the formulated media. For instance, potassium (K) plays a role in the transport of water, nutrients, and carbohydrates throughout plant tissues. As a result of this, enzyme activity in the plant impacts the creation of proteins, starches, and ATP. Photosynthesis can be slowed or sped up by altering the amount of ATP produced towards the plant's overall productivity (Oosterhuis *et al.*, 2014), calcium supports the enzymatic functions of the media amendments, and magnesium contributes significantly to the photosynthetic activities of the crop's agronomic performance.

***3.4.*0 Relationship between media quality, nutritional quality, and mineral uptake**

Vegetables supply essential dietary nutrients to meet human daily nutritional requirements and confer health benefits, reducing our risks of acquiring common lifestyle-associated diseases (Sarker and Oba, 2018). Some of these nutritional components include phenolic, vitamins, and antioxidants. The phenolic compounds are responsible for color, bitterness, acerbic taste, flavor, odor, and antioxidant properties in vegetables. The antioxidant compounds are accountable for preventing the oxidation of biomolecules by free radicals, limiting various human diseases' onset (Khanam *et al.*, 2012).

The present study sought to delineate the relationship between the media quality of RD-based amendment with crop agronomic performance and nutritional qualities such as the protein content of the leaves, phenolic content, antioxidant capacity, saturated fatty acid (SFA), Mono-

saturated fatty acid (MUFA), and minerals including Ca, K, Fe, Mn, Mg, etc. The strong positive significant relationship of MUFA with FW, NL, and TBM, could be attributed to the quality of the natural media amendment. For instance, it has previously been reported that natural growing medium amendments are made up of humic and non-humic substances that can provide plants with fulvic acid, humic acid, mineral nutrients, free amino acids, phytohormones, and a variety of other compounds necessary for plant growth and development.(Lazcano and Domínguez, 2011)
RD application as soil amendment improves cation exchange capacity and releases K, P, and Ca (Gillman *et al.*, 2002). Plant growth and development and favorable environmental conditions tend to stabilize the ratio of galactolipids to phospholipids, which can affect PUFA content in the tissue of the plants produced in the natural media amendment (Moellering and Benning, 2011).

Nutrient status and quality of crops are determined by phytochemicals, as are consumer perceptions of health advantages. (Abbey *et al.*, 2018b).

Principal component analysis and correlation showing the relationship between crop agronomic performance, soil and nutritional quality of lettuce crop grown in rock dust-based amendments

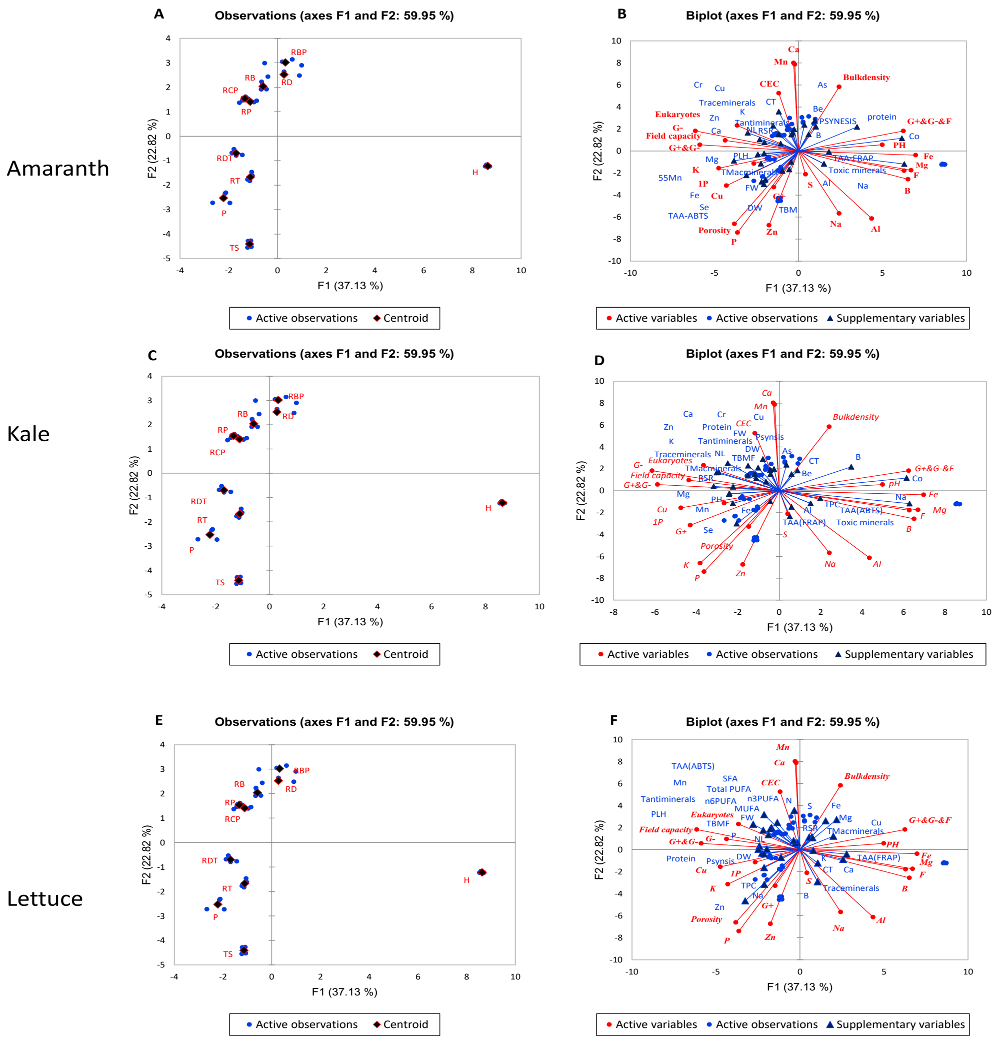


Figure 3.12: Principal component analysis (PCA) and correlation matrix showing the relationship between crop agronomic performance, soil, and nutritional quality of lettuce crop grown in rock dust-based amendments under a controlled environment system (A-B) Amaranth (A) Observation plot showing segregation of the rock dust-based treatments and control based on the centroids on F1 and F2 axis; and (B)Biplot showing a relationship between different observations crop production, soil quality, and nutritional quality of amaranth. (C-D) Kale (C) Observation plot showing segregation of the rock dust-based treatments and control based on the centroids on F1 and F2 axis; and (D) Biplot showing the relationship between different observations crop production, soil quality, and nutritional quality of kale. (E-F) Lettuce (E) Observation plot showing segregation of the rock dust-based treatments and control based on the centroids on F1 and F2 axis; and (F) Biplot showing the relationship between different observations crop production, soil quality, and nutritional quality of kale. RSR= Root shoot ratio, N=nitrogen, G+=Gram positive bacterial, PUFA=Poly unsaturated fatty acid, DW=Dry weight, TAA(ABTS)=Total antioxidant,n3PUFA=Omega 3, TBMF=Total biomass of fresh weigh, PLH=Plant height, SFA=Saturated fatty acid, CEC=Cation exchange capacity, Mn ppm=Manganese content in the leaves, Psynesis=Photosynthesis, Zn=Zinc content in the leaves, S=Sulphur content in the media and P=Phosphorus content in the media. R-values represent Pearson Correlation Coefficients, p= 0.05, n=4 per replicate of each sample.

Table 3.5: Correlation of kale agronomic performance, nutrient quality, and composition of rock dust-based amendment

	Variables	*Bulk density*	*G+&G-&F*									
QUADRANT 1 (RBP, R.D.)	*G+&G-&F*	**-0.808**										
	PH	**-0.810**										
	Co	**0.801**	**-0.894**									
QUADRANT 3 (RDT, RT, P, TS)	**Variables**	**PLH**	*P*	*K*	*Zn*	*Cu*	**Fe**	*Porosity*				
	1P	**0.673**										
	K	**0.607**	**0.904**									
	Zn	**0.659**	**0.809**	**0.947**								
	Cu	**-0.710**	**-0.815**	**-0.937**	**-0.966**							
	G+	**0.590**	**0.714**	**0.735**	**0.796**	**-0.867**						
	Fe		**-0.610**									
	Mn						**0.796**					
	Se							**0.681**				
QUADRANT 4 (RBP,RP,RCP)	**Variables**	**NL**	**TBMF**	*G+&G-*	*G-*	*Eukaryotes*	*Mn*	**TMacminerals**	**Traceminerals**	**RSR**	*Ca*	*Protein*
	G-	**-0.702**										
	Eukaryotes	**-0.798**	**-0.607**		**0.715**							
	CEC			**-0.903**								
	Mn	**-0.640**			**0.725**	**0.935**						
	TMacminerals			**-0.734**		**0.627**	**0.807**					
	K			**-0.699**		**0.689**	**0.851**	**0.994**				
	Ca								**0.999**			
	Tantiminerals									**-0.643**		
	DW	**0.626**	**0.614**									
	TBMF	**0.821**										
	Cu											**-0.692**
	Zn										**0.650**	

Correlation matrix based on quadrants in figure 5 showing the relationship between agronomic performance ,soil,and nutritional quality .G+=Gram positive bacterial in the media ,G-=Gram negetive bacterial , Eukaryotes= Bacterial level in the media ,Mn ppm=Manganese content in plant leaves ,Protein= Protein content of plant leaves ,TMacrominerals= Total Macrominerals in the plant leaves , Ca ppm= Calcuim level in the plant leaves ,Calcium= Calcium level in the media,Zn=Zinc contnet in the plant leaves ,Tracemineral=Total trace element in the lant leaves ,Be ppm=Beryllium content of the plant leaves,B ppm= Boron content in the plant leaves Se=Selenium content of the plant leaves , Zinc(mgL-1)= Zince level in the media ,Copper (mg L-1)= Copper content in the media , P=Phosphorus contnet in the media,K(ppm)=Potassium contnet in plant leaves measured in part perbillion . R-values represent Pearson Correlation Coefficients, p= 0.05, n=4 per replicate of each sample. 3

The **media compositions** are in italics :(*Bulk density, Porosity, Field capacity ,Eukaryotes, G-=negative, G+=gram positive ,F=fungi, 1P=protozoa, G+&G-=gram positive and negative bacterial, pH=soil pH, CEC=cation exchange capacity P=Phosphorus, K=Potassium, Ca=Calcium, Mg=Magnesium S=Sulphur , Zn=Zinc, Cu=Copper Na=Sodium, Fe=Iron, B=Boron, Mn=Manganese, Al=Aluminum.*

The agronomic performance and nutritional quality of the crops are in bold :(NL=number of leaves, TBMF=Total biomass of fresh weight, RSR=Root shoot ratio, TMacmineral =Total macrominerals of the leaves, Dw=Dry weight of the leaves, ca=Calcium ,Zn=zinc ,Cu=Copper, Traceminerals and protein

4

Table 3.6: Correlation of lettuce agronomic performance, nutrient quality, and composition of rock dust-based amendment

QUADRANT 1 (RBP,RD)	**Variables**	*pH*	**RSR**	**TMacminerals**	**S**	*Bulkdensity*								
	S	0.847												
	Bulkdensity	0.921	0.881	0.719	0.714									
	G+&G-&F	-0.889				-0.808								
QUADRANT 2 (H)	**Variables**	**CT**	*Al*	**B**										
	K	0.984												
	Ca		-0.998											
	TAA(FRAP)			0.858										
QUADRANT 3 (RDT,RT,P,TS)	**Variables**	**DW**	**Psynsis**	*Porosity*	*Cu*	*1P*	*G+*	*Zn*						
	Cu	-0.640	-0.636	-0.628										
	1P	0.921	0.567		-0.757									
	G+			0.750	-0.713									
	P			0.693			0.647							
	Na			0.615	-0.901	0.600	0.681							
	K	0.566	0.521					0.721						
QUADRANT 4 (RB,RP,RCP)	**Variables**	**PLH**	**NL**	**FW**	**TBMF**	*Field capacity*	*Eukaryotes*	*G+*	*G+&G-*	*CEC*	**P**	**Mn**	**SFA**	**n3PUFA**
	FW	0.648												
	TBMF			0.975										
	G+		0.685			0.589								
	G+&G-		-0.608					-0.928						
	CEC		0.656					0.965	-0.903					
	Tantiminerals											0.937		
	N			-0.691	-0.591						0.843			
	P	-0.717		-0.857	-0.796									
	Mn						0.671		-0.622					
	SFA		0.753											
	n3PUFA		-0.709	-0.680	-0.736	-0.602							-0.824	
	Total PUFA		-0.750	-0.683	-0.698			-0.691	0.603	-0.683	0.646		-0.871	0.891
	TAA(ABTS)						-0.638					-0.578		
	MUFA		0.650	0.769	0.825									

Correlation matrix based on quadrant showing the relationship between agronomic performance ,soil, and nutritional quality .RSR= Root shoot ratio,TPC=Total phenolic content of plant leaves, Eukaryotes= Bacterial level in the media TBMF=Total biomass of fresh weigh , FW=Fresh weight of plant,pH=level of acidity or alkalinity of the media, CEC=Cation exchange capacity, G+&G-&F= Gram positive, gram negative and fungi in the soil , Manganese content in the soil ,Psynesis=Photosynthesis, CT=Chlorophyll content of plant leaves, Co=Cobalt content in plant leaves ,As ppm= Arsenic content in plant leaves ,Cr=Chromium content of the soil, K(ppm)=Potassium content in plant leaves measured in part per billion ,. R-values represent Pearson Correlation Coefficients, p= 0.05, n=4 per replicate of each sample.

The media compositions are in italics *:(Bulk density, Porosity, Field capacity ,Eukaryotes, G-=negative, G+=gram positive ,F=fungi, 1P=protozoa, G+&G-=gram positive and negative bacterial, pH=soil pH, CEC=cation exchange capacity P=Phosphorus, K=Potassium, Ca=Calcium, Mg=Magnesium S=Sulphur , Zn=Zinc, Cu=Copper Na=Sodium, Fe=Iron, B=Boron, Mn=Manganese, Al=Aluminum)*

The agronomic performance and nutritional quality of the crops are in bold :(NL=number of leaves, TBMF=Total biomass of fresh weight, RSR=Root shoot ratio, TMacmineral =Total macrominerals of the leaves, Dw=Dry weight of the leaves, ca=Calcium, Zn=zinc ,Cu=Copper, Traceminerals proteins , SFA=saturated fatty acids, MUFA=Monosaturated fatty acids ,PUFA=Polysaturated fatty acids,1P=protozoa, TAA(ABTS)=Total Antioxidant content by ABTS method ,n3PUFA=Omega 3,n6PUFA=Omega 6 ,N=nitrogen content of crops , P=Phosphorus, Mn=Manganese

Table 3.7: Correlation of Amaranth agronomic performance, nutrient quality, and composition of rock dust-based media

Quadrant 1 (RBP, RD)	**Variables**	**Co**	*protein*	*Bulkdensity*						
	Co									
	As	0.810								
	pH	0.798								
	Bulk density		0.971							
	G+&G-& F		-0.793	-0.808						
Quadrant 3 (RDT,RT,P,TS)	**Variables**	*Porosity*	**PLH**	**FW**	**TBM**	*P*	*K*	*Zn*	*Cu*	**Mg**
	FW	0.737				**0.743**				
	TBM	0.801		0.913		**0.654**	**0.685**	**0.785**		
	TMacminerals		-0.588							
	Mn									0.865
	Se	0.681		0.626						
	TPC			0.601						-0.611
	K			**0.692**		0.904				
	Zn			**0.789**		0.809	0.947			
	Cu			**-0.787**		-0.815	-0.937	-0.966		
	DW							0.679	-0.592	
	Fe					-0.610				
	G+			0.79	0.678	0.714	0.735	0.796	-0.867	
Quadrant 4 (RB,RCP,RP)	**Variables**	**NL**	**CT**	**RSR**	**K**	*Eukaryotes*	*G-*	*G+&G-*	*Mn*	**RSR**
	K			0.822						
	Cr			0.579						
	Eukaryotes				0.689					
	G-					0.715				
	G+&G-		0.587	-0.810	-0.699					
	CEC		-0.678	0.612				-0.903		
	Mn			0.739	0.851	0.935	0.725			
	Ca									0.756

35 Correlation matrix based on quadrant showing the relationship between agronomic performance ,soil,and nutritional quality .RSR= Root shoot ratio, N=nitrogen ,G+=Gram positive bacterial ,PUFA=Poly
36 unsaturated fatty acid, DW=Dry weight,TAA(ABTS)=Total antioxidant,n3PUFA=Omega 3,TBMF=Total biomass of fresh weigh ,PLH=Plant heigt ,SFA=Saturated fatty acid,CEC=Cation exchange capacity,Mn ppm=Manganese content in the leaves ,Psynesis=Photosynthesis,Znppm=Zinc content in the leaves, S=Sulphur content in the media and P=Phosphorus content in the media.R-values represent Pearson Correlation Coefficients, p= 0.05, n=4 per replicate of each sample.

The **soil composition** are in italics :(*Bulk density, Porosity, Field capacity ,Eukaryotes, G-=negative, G+=gram positive ,F=fungi, 1P=protozoa, G+&G-=gram positive and negative bacterial, pH=soil pH, CEC=cation exchange capacity P=Phosphorus, K=Potassium, Ca=Calcium, Mg=Magnesium S=Sulphur , Zn=Zinc, Cu=Copper Na=Sodium, Fe=Iron, B=Boron, Mn=Manganese, Al=Aluminum*

3.4.1 Proposed mechanism of how RD-based media amendment influence crop performance and nutrient quality

To the best of our knowledge, this is one of the first studies to propose a mechanism (Fig.3.13) to explain how RD as a natural media amendment appears to improve growth media quality, agronomic performance and nutrient quality of horticulture crops as a cost-effective and sustainable approach for precious metals mining waste management to enhance food security. We have tested the RD-based growth media following development, assess the beneficial effects of RD on the developed potting growth media and its effects on vegetable growth, production, and produce quality under controlled environmental conditions. In the current study, we observed that the addition of RD to different growth media such as biochar, promix, compost, and topsoil improved the growth media's physicochemical and biological properties (Table 2.2 to 2.4 and Fig 3.13). The addition of RD to different growth media improved the nutrient availabilities, bulk density, porosity, field capacity, and microbial composition (Table 2.2 to 2.4 and Fig 3.13). With the low bulk density and high porosity, the packed clay particles of RD may have been a driving force resulting in an equal distribution of moisture and nutrients, creating a favorable habitat for soil microorganisms (Sung *et al.*, 2010). These properties might further enhance the mineralization process to increase nutrient availability in the rhizosphere. Such results are evident from a strong Pearson's correlation (r=-0.81) among BD and G+ & G- & F (Fig 3.13. /Table 3.5). The RD amendment in growth media appears to supply the macro and micronutrients to growing plants due to high levels of Ca, K, Mg, P, Cu, Fe, Mn, Mo, Ni, and Zn as reported by Arnott *et al.* (2021). Such effects are obvious in RB, RBP, RCP, and RP treatments, as depicted in (Table 2.2 to 2.4 and Fig.3.3 to 3.7). Also, the addition of RD in growth media resulted in improved microbial composition, which might further enhance mineralization or nutrient recycling to enhance nutrient availabilities. The enhanced mineral nutrients and superior soil health through improved microbial structure thus helped plants perform better in RD-based media amendments (Fig 3.5 to 3.7). Plants expressed improved agronomic performance as indicated by a positive but significant correlation (r = 0.68) between G+ bacteria and total biomass (Fig 3.3 to 3.7. / Table 3.7). This association could be due to the improvement of the environmental conditions in the growth media, which is provided by the addition of RD to enhance crop performance in producing high-quality yields. Significantly, taller plants, increased total biomass, fresh weight, improved root-shoot ratio, and a greater number of leaves were observed in RD-based media treatments (Fig.3.3 to 3.7). The vegetable plant's superior agronomic performance was also evident from a strong positive correlation between improved growth media properties and fresh weight, total biomass, photosynthesis (Table 3.5 to 3.7/Figs. 3.13). The improved nutritional quality also showed a positive correlation with improved media quality, as obvious from strong correlations between growth media properties and crop produce quality (Fig.3.13). The substantial relationships shown between media quality and crop agronomic performance and nutritional quality imply that RD-based media have superior bulk density, porosity, nutrient composition, and microbial composition. This improvement in the growth media resulted in crops with increased capacity to absorb nutrients from mineralized media and translocate them into superior biomass and bioaccumulation of the following nutrients: protein, MUFA, total antioxidant, and total macronutrients. These results suggest that RD, a low-value by-product of precious metal mining, appears to have unique properties in improving health status and fertility of growth media, boosting crop growth, yield, and nutritional quality.

The relation observed between the media quality and crop agronomic performance and nutritional quality supports the improvement in bulk density, porosity, nutrient composition, and microbial composition of RD based media. This resulted in crops having an enhanced capacity to absorb nutrients from the media mineralized by G+&G-&F and transform this into nutritional quality.

PROPOSED MECHANISM OF ROCK DUST-BASED AMENDMENT

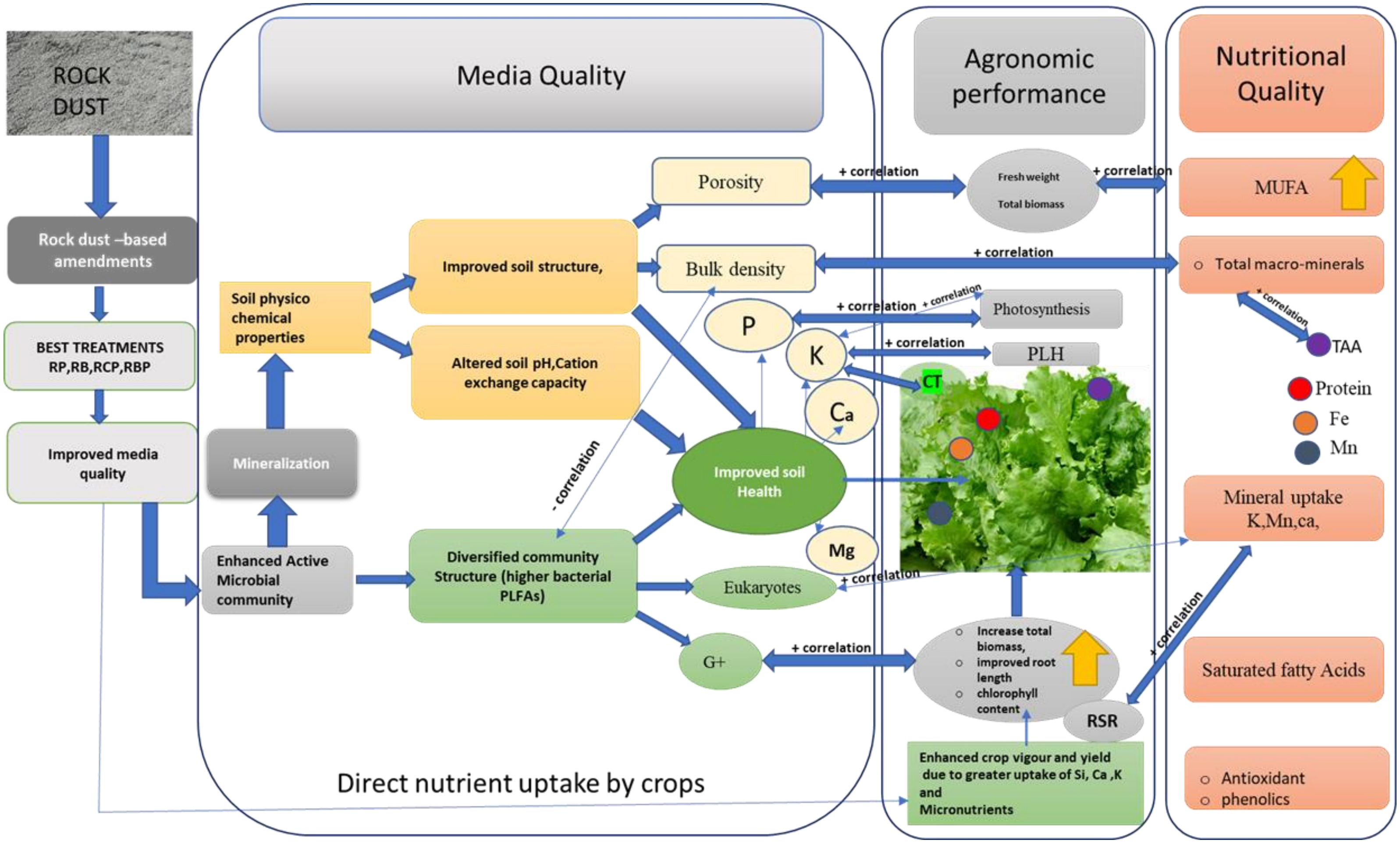


Figure3.13: Proposed Mechanism suggesting how RD-based amendments maybe influencing media quality, crop growth performance, nutrient uptake and vegetable nutritional quality.

The following media amendments (RCP, RBP, RB, and RP) were the best media based on their improved bulk density, nutrient and microbial composition. These media had enhanced active microbial community composition resulting in elevated levels of P, K, Ca, Mg available for crop utilization during growth. This was supported by the strong correlations (r=-0.81) between BD and G+&G-&F bacterial with an increase total biomass leading to improvement in agronomic performance. The strong association of porosity with fresh weight and total biomass as well as the nutritional quality of the plants suggest RD based media resulted in crops having an enhanced capacity to absorbed nutrients from the mineralized media for maximum yield. The RSR was directly correlated with the uptake of K, Mn and Ca improving the levels of these minerals and the crop nutritional quality. Collectively, the findings demonstrated RD based media contain improved physical (porosity, bulk density), chemical (P, K, Ca, Mg, CEC, pH) and biological (active G+ and Eukaryotes) composition. This resulted in vegetable crops with larger RSR impacting the ability to absorb more nutrients (e.g. K, Ca), increased chlorophyll, photosynthesis and total biomass (yield). The bulk density, active soil bacterial composition, porosity and RSR were highly associated with the enhanced crop nutritional composition. Specifically, porosity with the increased the MUFA content, bulk density with the increased total macronutrients and RSR with the enhanced K, Mn and Ca uptake and bioaccumulation in the crops produced. This work present for the first time an hypothesize pathway via which RD appears to improve growth media quality or health influencing horticultural crop growth performance and phytonutrient composition. TAA=Total antioxidant activity, MUFA=Monosaturated fatty acid=Total biomass of crop, PLH=Plant heights=Root shoot ratio, K=Potassium, and P=Phosphorus.

3.5 Conclusion

In this study, we evaluated the potential of RD as a natural media amendment to grow horticultural crops under controlled environmental conditions and assess its performance in crop growth, yield, nutritional quality, and sensory evaluation. Herein, we have shown that RD can serve as potential soil amendment to improve growth media, crop growth, and nutritional quality.

The findings of this study showed that RD-based growth media amendments are effective at improving crop agronomic performance and the nutritional composition (fatty acids, protein, minerals, and antioxidants) in horticultural crops grown under controlled environmental conditions. Out of the ten different media formulations, RCP, RBP, RB, and RP resulted in improved plant performance (biomass, chlorophyll content, plant height, dry weight, and the number of leaves). The improved agronomic performance is strongly associated with improved growth media quality for example: There was a strong positive correlation of porosity with fresh weight and total biomass of plant. Moreover, RD amended growth media displayed higher protein content in amaranth crop, total phenolic contents, and total antioxidant content than non-RD growth media. These results are supported by strong positive correlations of improved nutritional quality with the media quality. For example, a strong positive correlation of BD with total macro minerals of plant tissue. Additionally, microbial composition showed strong positive correlation with the following; total biomass, root growth, K, Mn, and Ca in the leaves.

The RCP media stood out as the best media for amaranth crops with the highest protein content. According to the sensory evaluation analysis, the lettuce crop cultivated in RB ranked best in overall appearance. The four media amendments (RCP, RBP, RB, and RP) had low bulk density, high porosity, and increased microbial composition. These qualities might have provided a favourable environment for microbial interactions, water retention, root development, mineralization, and crop growth. The RD-based amendment enhanced the media quality through improved microbial composition. The active microbial communities were responsible for mineralization of nutrients, leading to nutrient availability to improve soil conditions for root growth, plant health, yield, and nutritional quality. The increased porosity supported a rationale for more available oxygen and improved water holding capacity following amendments with RD in the best performing media. This is supported by a strong positive correlation of the root with fresh weight, and total biomass leading to enhanced nutritional quality.

This study demonstrated a strong relationship between media quality with crop agronomic performance and nutritional composition through a proposed mechanism. The media quality of low BD showed a strong negative correlation with the microbial composition, depicting that low BD led to an improved activity of the microbial composition in the RD-based amendments which appears to improve mineralization, leading to improved crop growth and yield. Furthermore, the low BD of the media showed a positive correlation with the total macro-minerals in the plant tissues.

It is widely acknowledged that diet impacts population health and that there are strong links between diets high in vegetables and lower risks of developing common lifestyle-related illnesses. It has been suggested that using natural media amendments, especially volcanic minerals, could be a valuable approach to produce vegetables with increased nutritional quality to reduce health-related diseases. Hence, based on these results, we conclude that these RD-based amendments' rates (RCP, RBP, RB, and RP) with other soil measurements could be a suitable strategy not only to develop a natural media amendment ideal for improving crop productivity, but could also address environmental issues related to sustainable agricultural production and waste management. The use of RD amendment is relevant to the mining and agricultural sectors because it could provide alternative for by-product disposal and serve as a natural media amendment for the horticultural industry to increase productivity as well as produce nutrient-rich crops, thereby reducing cost and excessive use of synthetic fertilizers.

Further research should be conducted with different crop varieties other than the three crops used in this experiment. An optimization of the following RD based media amendments (RCP, RB, RBP, and RP) would be needed before recommending to farmers for production.

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CHAPTER 4

4.0 Summary and Conclusion

The aim of this research project was to evaluate the potential of RD as a local natural media amendment or alternative nutrient source to boost vegetable production and quality in a controlled environmental condition in Newfoundland and Labrador (NL). The specific objectives were to 1) analyze the physicochemical properties of RD for growth media formulation, 2) evaluate the effect of RD amended growth media on the agronomic performance of kale, amaranth, and lettuce in a controlled environmental condition, 3) evaluate effect of RD amended growth media on the nutritional quality and sensory evaluation of the produced vegetables, 4) investigate the mineral composition of vegetable plant tissue and 5) determine the relationship between the RD amended growth media quality, the agronomic performance of crops and nutritional quality.

Chapter 1 is composed of a comprehensive review of literature on mining by-products (RD), effects on the environment, and the potential as natural media amendment for the production of vegetable crops. This includes success findings and contradictions of previous researchers.

In **chapter 2,** the research findings demonstrated that RD and RD-based amendment had low bulk density below the plant root restriction threshold with high porosity. The RD particles were gravel-free and below the 2-millimeter diameter of particle size distribution with 50% sand, 30% clay, and silt 20% of fine particle size. The RD has a similar particle size as clay and silt, which demonstrate the ability to store and hold cation, indicating the potential of plant nutrient availability. To confirm the above indications, our analysis of the RD samples revealed the availability of sodium (Na^+), ammonium (NH_4^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), iron (Fe^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}) and manganese (Mn^{2+}) which are beneficial to plants. Furthermore, the study found a diverse, active microbial community in RD-based amendments, which could be responsible for the mineralization of RD to make the nutrients available for plant growth. The present study demonstrated an improvement in the physiochemical properties of the formulated RD-based amendments. The RD-based amendments were found to contain some essential nutrients required for plant growth and could be a potential source as a natural media amendment for vegetable production.

Chapter 3 highlighted the evaluation of the formulated RD-based growth media as natural media amendments to grow horticultural crops under controlled environmental conditions and assess their performance in crop growth, yield and nutritional quality, and sensory evaluation.

Herein we proposed an environmentally sustainable approach to use RD as a growth media amendment due to the presence of minerals and positive effects on growth media quality. The results depicted that RD amendments in different growth media resulted in improved the growth of horticultural crops including lettuce, kale, and amaranth. The crops grown in RD amendment performed better or equal to other non-RD media with improved chlorophyll content, number of leaves, fresh biomass, dry weight, and root shoot ratio. The higher performance is associated with better growth media quality supported by strong positive correlations among growth performance. Moreover, RD amended growth media displayed higher crop nutritional quality in terms of protein, total phenolic contents, and total antioxidant content than non-RD growth media. These results are supported by positive correlations of improved quality with media quality and crop performance. Overall, RD improved growth media quality, plant agronomic performance, and product quality in the following RD-based amendments RCP, RBP, RB and RP. It could be concluded that adding RD as a growth media amendment has positive effects on plant performance and quality due to improved growth media.

This study is relevant to the mining and agricultural sectors because it provides alternative by-product disposal and a natural media amendment for the horticultural industry to produce nutrient-rich crops, thereby reducing the cost and excessive use of synthetic fertilizers.

6.1 **Future work**

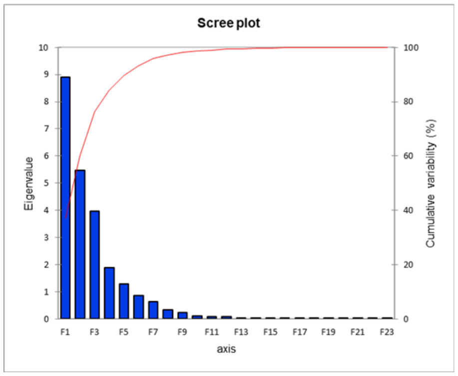
This study cultivated only one variety of each crop, so it will be prudent to experiment using the different formulated RD-based amendments for growing different varieties of the same crops in controlled environmental conditions.

An optimization of the best media amendments would be needed before recommending to farmers the production of crops.

Supplementary data for figure 3.12

Eigenvalues:

	F1	F2	F3	F4	F5
Eigenvalue	8.911	5.477	3.958	1.876	1.292
Variability (%)	37.127	22.820	16.494	7.818	5.381
Cumulative %	37.127	59.948	76.441	84.259	89.641

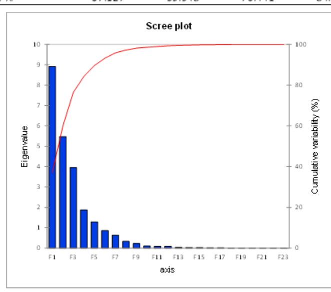


Eigenvalues: Lettuce

A

	F1	F2	F3	F4	F5
Eigenvalue	8.911	5.477	3.958	1.876	1.292
Variability (%)	37.127	22.820	16.494	7.818	5.381
Cumulative %	37.127	59.948	76.441	84.259	89.641

B



Eigenvalues: Amaranth

A

	F1	F2	F3	F4	F5
Eigenvalue	8.911	5.477	3.958	1.876	1.292
Variability (%)	37.127	22.820	16.494	7.818	5.381
Cumulative %	37.127	59.948	76.441	84.259	89.641

B