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Influence of soil depth on seedling growth and development of *Amaranthus viridis* L. after amendment with organic fertilizer

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Abstract. The implications of applying organic fertilizers made from dried cow dung and food waste on the seedling development and growth of *Amaranthus viridis* at different soil horizons was examined in the current study. The experiment was set up in a completely randomized design, with 6 profile depths x 3 fertilizer treatments x 3 replicates each, totaling 54 bowls. The set up was left for 5 weeks. The findings demonstrated that cow dung manure provided adequate plant nutrients for enhancing seedling growth *A. viridis*. Although 0.05 g seeds of *A. viridis* was sown in the bowls, emergence capacities under varying soil conditions differed. Amending the soils with food waste manure hampered emergence capacities of the test plant. There were only six (06) sprouts at the first week compared to 46 in the control. Despite the observation that soil horizon and organic fertilizer application had no discernible effect on the root length of 5 - week - old seedlings (which varied between 1.3 cm and 2.3 cm), the use of manure was associated with a decrease in foliar chlorosis incidence. Without manure, foliar chlorotic incidence was 58 % in the 61 – 75 cm soil profile depth, compared to 35.0 – 35.2 % when soils were amended with either cow - dung or dried food waste manure. Ultimately, while deeper soil horizons initially posed challenges for growth, soil amendment successfully alleviated these unfavorable conditions.

Keywords: *soil horison, food waste manure, cow dung manure, nutrient availability, foliar chlorosis*

Introduction

Soil composition is dynamic and varies significantly across different levels due to various factors, including organisms, plants, microbes, and human activities (Kumar, 2020). This variability affects soil structure, fertility, and overall productivity. Human activities, such as deforestation, intensive farming, and urbanization, have profound impacts on soil health. Urbanization, in particular, has led to widespread soil degradation, erosion, and loss of arable land.

The consequences of human population expansion and urbanization on farmland are alarming. Farmlands intended for agricultural use have been rapidly taken over by estate developers and mining support businesses, resulting in soil excavation, environmental harm, and decreased food production (Oluwatosin and OjoAtere, 2001). This trend is exacerbated by the growing demand for housing, infrastructure, and natural resources. As a result, fertile soils are being irreversibly damaged, threatening food security and sustainable development.

Ensuring sustainable food production and soil management presents formidable challenges globally. The continuous expansion of the world's population is expected to intensify the strain on already burdened food systems (United Nations, 2020). Additionally, the increasing conversion of land due to urbanization, which involves significant topsoil removal for construction, contributes substantially to soil degradation and erosion (Wainaina *et al*., 2022; Pimentel, 2006).

Healthy soil is essential for the maintenance of vital ecosystem services, such as the filtering of water and the sequestration of carbon (Gong & Chen, 2011; Lal, 2015). However, the processes of soil excavation and erosion can severely compromise soil quality and fertility, negatively affecting agricultural productivity and disrupting these critical ecosystem services (Zhao *et al*., 2021; Kumar, 2020). In Nigeria's peri-urban areas, the practice of unmanaged soil excavation has resulted in substantial environmental deterioration, a decline in biodiversity, and adverse impacts on the livelihoods of local communities (Wainaina *et al*., 2022).

This study aims to address the urgent problems of soil degradation, diminished crop productivity, and the need for environmental sustainability by examining how different soil horizons influence crop development, with a specific focus on *Amaranthus viridis*. Specifically, the research will investigate the effects of varying soil horizons on the growth, yield, and nutritional value of *A. viridis*, a crop of significant importance for food security and nutrition in numerous communities. Furthermore, by evaluating the potential of organic fertilizer formulations derived from common household waste, this research seeks to contribute to the enhancement of agricultural output, the promotion of longterm food security, and the strengthening of ecosystem resilience.

Materials and methods

Study location

This research was conducted at the University of Benin's Ugbowo campus, specifically near the Department of Plant Biology and Biotechnology's screen house, located in Benin City, Edo State, Nigeria.

Experimental design

The study employed a completely randomized design (CRD) to investigate the effects of different soil profiles and organic amendments on crop development. The treatments consisted of soils from different profiles amended with cow dung manure (CDM), soils from different profiles amended with food waste manure (FWM), and a control group without any amendments. Each treatment was replicated three times.

Soil sampling

Soil samples were collected from three random farm locations in Ovia North-East Local Government, Edo State, Nigeria (Figure 1). The sampling area was carefully selected, and surface litter was removed. When soils were collected, all soils were pooled to obtain a composite sample, which was thereafter used for the experiment.

For profile soil sampling, four profile pits were dug using a shovel and digger, and composite sampling was carried out to mix the same horizon from different pits. Samples were taken from the first horizon to avoid contamination.

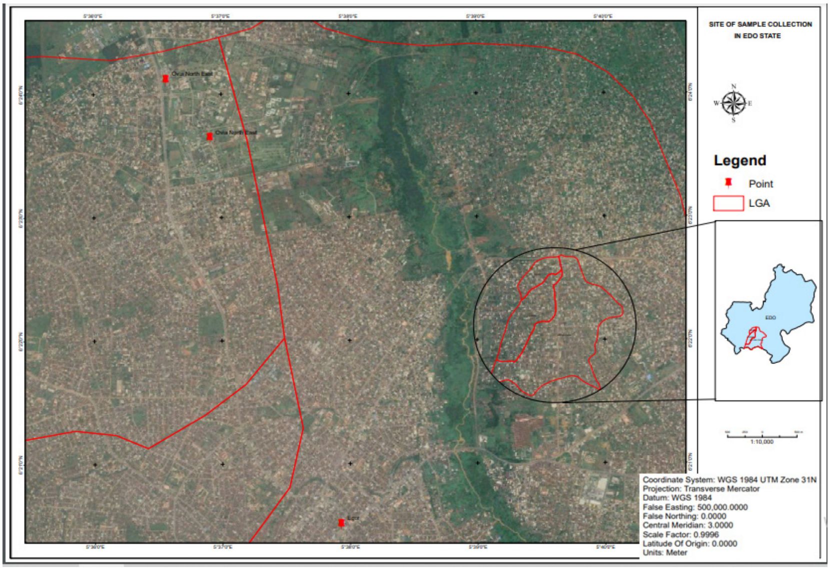


Figure 1. Map of soil collection locations

Each soil horizon was extracted and transferred into labelled nylon bags. Soil samples were collected from 0 to 75 cm depth, with 15 cm intervals, except for the topmost soil (0-10 cm and 11-15 cm). This resulted in six samples each, representing different horizons within the soil profile (Figures 2 and 3).

(a) **(b)**

Figure 2. Excavating one of the pits to obtain soil samples from various profile depths

(a) **(b)**

Figure 3. Image capturing the pit and its profiles

Development of organic fertilizers

This section outlines the procedures for developing two organic fertilizers used in the study.

Cow dung manure

Dried cow dung was sourced from the Aduduwa Cattle Market in Edo State. The dung was sun - dried to constant weight to ensure optimal moisture removal and readiness for use.

Dry food waste manure

The development of dry food waste manure (FWM) involved several key steps. Initially, food waste was collected from various locations, including Ekosodin and Uselu markets in Benin City, as well as local vendors. The collected waste was then sorted to separate different components. The composition of the dried food waste consisted of various organic materials, including orange peels (1800 g), onion peels (500 g), plantain peels (1000 g), pineapple peel (400 g), watermelon (200 g), yam peels (300 g), potato peels (180 g), vegetables (300 g), eggshells (50 g), and dried leaves (200 g).

The food waste components underwent further processing to create the FWM. Each component was sun - dried to constant weight for 30 days to ensure optimal moisture removal. The dried components were then blended into a fine powder using a local mill. Finally, the powdered components were mixed together to create a homogeneous blend. This resulting FWM, along with CDM, was used as an organic fertilizer to amend the soil samples in the experiment, providing a nutrient - rich medium for plant growth.

Separation into bowls

The soil from each horizon was divided among three bowls, labeled A, B, and C, and then replicated. This resulted in a total of 54 bowls, based on the experimental design: 6 profile depths × 3 fertilizer treatments × 3 replicates each. The bowls, with a total surface area of 304.84 cm^2, were modified to prevent waterlogging. Five evenly spaced perforations were made in the bottom of each bowl using a nail, allowing for adequate percolation and drainage.

Treatment application

This research utilized 54 bowls, divided into three categories: A, B, and C. Categories A and B consisted of six bowls each, with different soil profiles mixed with CDM and dry FWM, respectively. Category C comprised 18 unamended soil profiles as controls. For the amended bowls (A and B), organic fertilizers were added on a weight/weight basis at a 10 % w/w concentration, with 1.6 kg of CDM or FWM added to 16 kg of soil dry weight per bowl.

Sowing

Following a one - week period, *A. viridis* seeds were broadcast - sown at 0.05 g per treatment. To facilitate even seed distribution and prevent overcrowding, this method was chosen. The bowls, with a surface area of 304.84 cm^2, received 1000ml of water after seeding and were then moved to their permanent locations. The resulting seed application density was 0.048 g per 304.84 cm^2, equivalent to 0.164 mg/cm^2.

Cultural practices and parameter measurement

The bowls were watered every two days for the first two weeks, then daily thereafter. Regular hand weeding was performed at intervals. To assess plant growth and development, several parameters were measured: plant height, chlorosis, leaf color, number of leaves, root length, and biomass. Specifically, plant height was measured using a meter rule on an index plant in each bowl. Chlorosis was evaluated by observing yellowing foliage as plants aged. Leaf color was assessed visually using a physical color namer map. The number of leaves in each bowl was counted and recorded. Root length was measured by carefully removing the index plant from each bowl and using a meter rule.

Determination of soil physico - chemical characteristics

Soil samples were collected at 3 random areas within pooled random depths (0 – 15 cm) within the area where soil was obtained, just before determination of plant identification and determination for soil seed bank assessment. Top soil was collected. Soils were analyzed for soil physico - chemical parameters according to methods described by Hanway and Heidel (1952); Metson (1961); Nelson and Sommers (1982); APHA (1985).

Isolation of bacterial and fungal isolates

Bacterial and fungal isolates were isolated using established microbiological protocols (Cheesebrough, 2000). Soil samples from each treatment underwent serial dilution and plating on selective media (nutrient agar for bacteria and potato dextrose agar for fungi). Following incubation at optimal temperatures (28 °C for bacteria and 25 °C for fungi) for 24 - 48 hours, colonies were counted and isolated based on distinct morphological features. Isolated microorganisms were purified through successive subculturing and preserved on agar slants at 4 °C for subsequent identification and analysis (Cheesebrough, 2000).

Data analysis

Results are reported as means of three replicates. Single- - factor analysis of variance (ANOVA) was employed to evaluate treatment effects. Statistical analysis was performed using IBM SPSS Statistics version 23 and Paleontological Statistics (PAST) software version 2.17c, where necessary, to assess significant differences between treatments and provide a comprehensive understanding of the data.

Results

The microbial populations in the organic fertilizers used in the study, analyzed just prior to soil application, are presented in Figure 4. Notably, the bacterial counts differed significantly between the two fertilizers. The dry FWM harbored a mean bacterial count of 6.50 ± 2.12 x 10^4 CFU/g, whereas the CDM had a significantly higher count of 11.75 ± 1.06 x 10^4 CFU/g. In contrast, fungal counts were relatively similar, with FWM exhibiting 1.07 ± 0.42 x 10^4 CFU/g and CDM showing 0.93 ± 0.36 x 10^4 CFU/g.

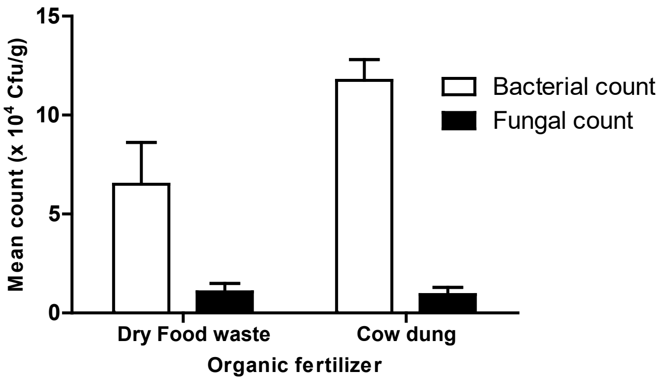


Figure 4. Bacterial and fungal counts in the materials used as organic fertilizer just before application to soil

The culturable bacterial and fungal composition of organic fertilizers used in the study, analyzed just before soil application, is presented in Table 1. The comparative analysis revealed a higher diversity of bacterial isolates in cow dung compared to dried food waste. Specifically, *Proteus mirabilis* was the predominant bacterial isolate in cow dung. In contrast, *Serratia marcescens* emerged as the dominant bacterial species in dried food waste. Regarding fungal composition, *Aspergillus fumigatus* and *Rhizopus stolonifer* were the predominant species in the organic fertilizers. Nutrient analysis, illustrated in Figure 5, showed significant differences between the two organic fertilizers. Cow dung exhibited a higher nitrogen content (12.43 %) compared to dried food waste. Conversely, dried food waste contained more potassium (36.34 %) than cow dung (28.12 %). These variations in microbial and nutrient composition may influence soil fertility and plant growth.

Table 1. Culturable bacterial and fungal composition of materials used as organic fertilizer just before application to soil

Isolates	Cow dung	Dry Food waste
Bacterial Isolates		
Bacillus subtillis	-	+
Proteus mirabilis	+++	-
Serratia marcescens	+	+++
Escherichia coli	+	+
Pseudomonas aeruginosa	+	-
Klebsiella oxytoca	+	-
Fungal Isolates		
Aspergillus niger	-	+
Aspergillus fumigatus	+	+
Rhizopus stolonifer	+	+
Trichoderma sp.	+	-
Penicillium sp.	-	+

Legend: + present; - absent

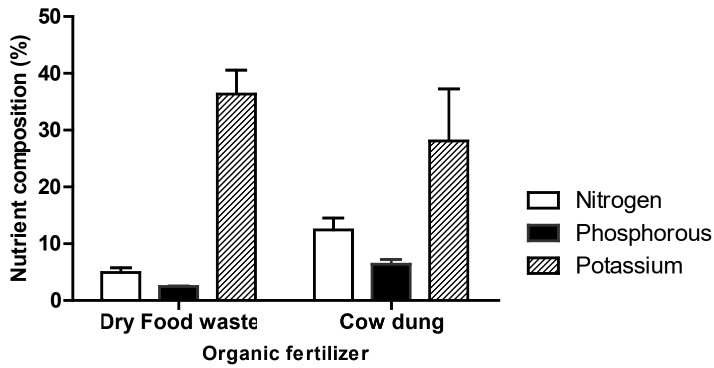
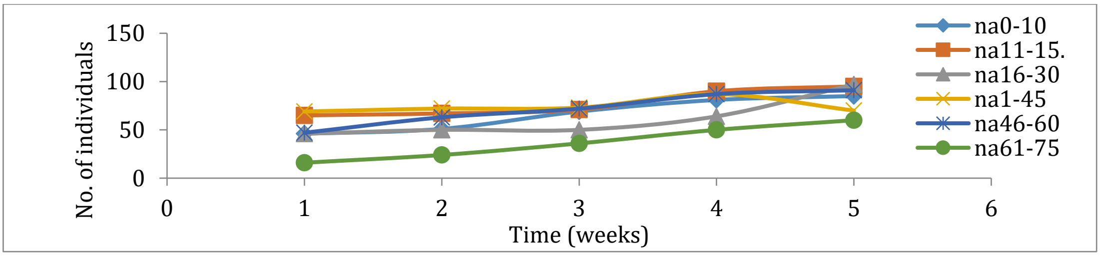


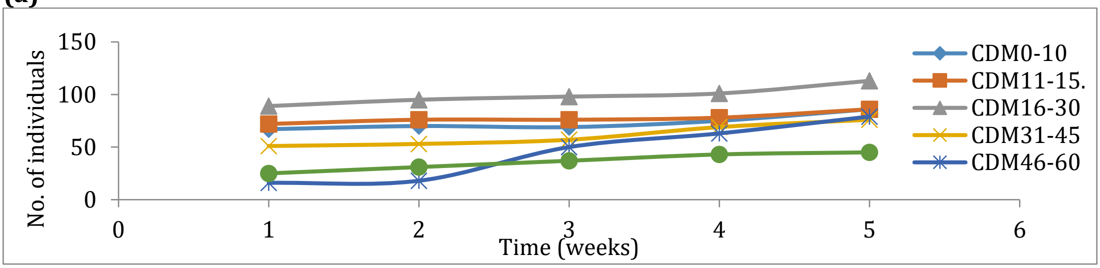
Figure 5. Nutrient composition of materials used as organic fertilizer just before application to soil

The emergence capacity of *A. viridis* seeds varied significantly under different soil conditions, as shown in Figure 6(a - c). Initially, 0.05 g of seeds were sown in each bowl. In unamended soils, collected from a depth of 0 - 10 cm, seed emergence was moderate, with 46 sprouts observed within the first week of observation. This number increased to 69 sprouts by the third week, indicating continued germination and growth. In contrast, amending the soil of the same depth with CDM resulted in enhanced seed emergence, with 67 sprouts recorded in the first week (Figure 3).

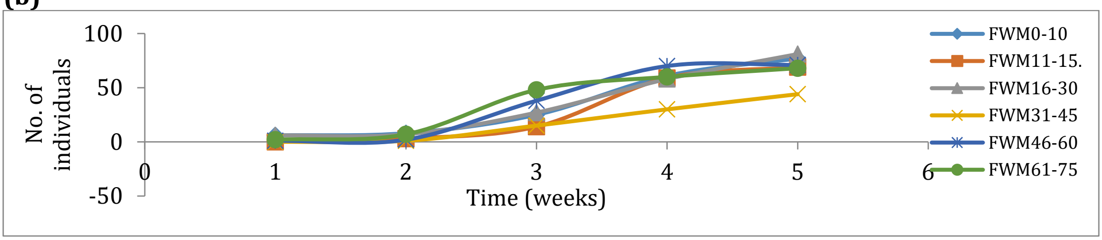
However, amending the soil with FWM had a detrimental effect on seed emergence. After the first week, only six sprouts emerged in FWM - amended soil collected from 0 - 10 cm depth, and merely one sprout was observed in FWM - amended soil collected from 46 - 60 cm depth. This suggests that FWM may inhibit initial seed germination. Nevertheless, by the third week, rapid growth was observed in FWM - amended soils, indicating potential for delayed but accelerated development (Figure 6).



(a)



(b)



(c)

Figure 6. Amount of sprouts of *Amaranthus viridis* sown from 0.05 g seeds by broadcast on soils collected at different depth profiles 0 - 10, 11 - 15, 16 - 30, 31 - 45, 46 - 60 and 61 - 75cm respectively and amended with food waste manure (FMW), cow dung manure (CDM), and unamended (na).

Figure 7. Plants grown on soils obtained from the various horizons

Figure 8 illustrates the growth of *A. viridis* seedlings in soils of varying profile depths at 5 weeks post - sowing. Notably, unamended soils supported the growth of 85 individual seedlings from 0.05 g of seeds. In contrast, soils amended with dried CDM exhibited depth - dependent effects. At the 0 - 10 cm depth, CDM - amended soils yielded 86 sprouts, while the 31 - 45 cm depth profile produced 97 sprouts. Remarkably, the same depth profile with CDM amendment resulted in 113 sprouts, indicating optimal growth conditions.

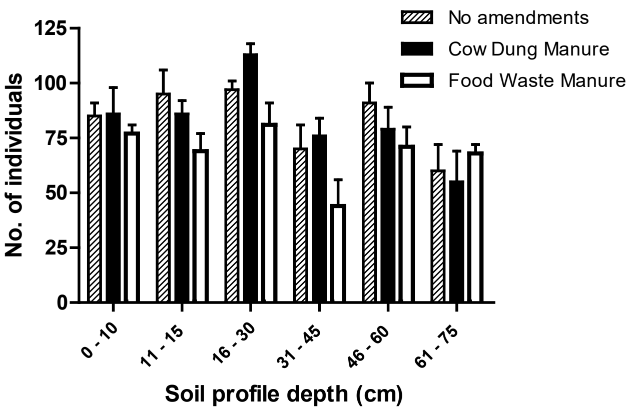


Figure 8. Number of individual *Amaranthus* seedlings grown in soils of differing profile depth at 5 weeks after sowing and soil amendments

The impact of soil depth on seedling growth was further underscored by the poor performance of seeds sown in deeper profiles (61 - 70 cm), regardless of amendment type. This depth range yielded only 55 - 68 sprouts, suggesting potential limitations in soil fertility, aeration, or moisture retention. In contrast, shallower depths (0 - 45 cm) demonstrated improved growth, particularly when amended with CDM.

Figure 9 illustrates the percentage of *A. viridis* seedlings with chlorotic foliage, indicating potential stress incidence. Notably, plants in deeper soil profiles (61 - 75 cm) exhibited higher foliar chlorosis (58 %) compared to those near the surface. However, manure application significantly reduced chlorotic incidence, with CDM and FWM amendments lowering rates to 35.0 - 35.2 %.

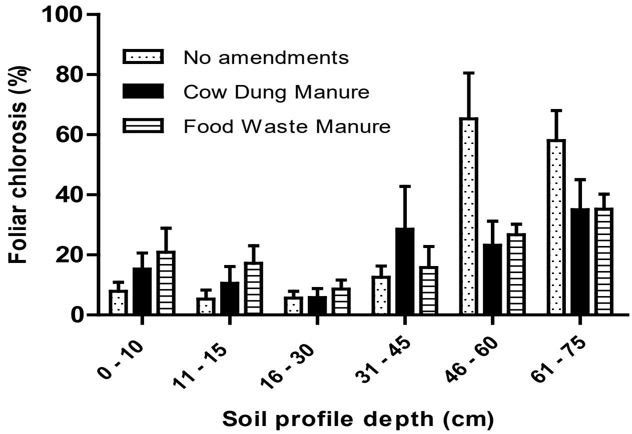


Figure 9. Percentage of leaves with chlorosis at 5 weeks after sowing and soil amendments.

The impact of soil amendments on seedling growth was further investigated. Without manure, leaf production remained stagnant (4 - 6 leaves per plant) across various soil profile depths. In contrast, adding CDM increased leaf production to 7 - 10 leaves per plant (Figure 10). This enhancement underscores the benefits of organic fertilizers on plant development. Root length, measured at 5 weeks, remained relatively consistent (1.3 - 2.3 cm) across soil horizons and fertilizer treatments (Figure 11). This suggests that root growth was not significantly influenced by soil depth or organic fertilizer application. Additional visual documentation of the experiment's setup at 3 weeks is provided in Figure 7.

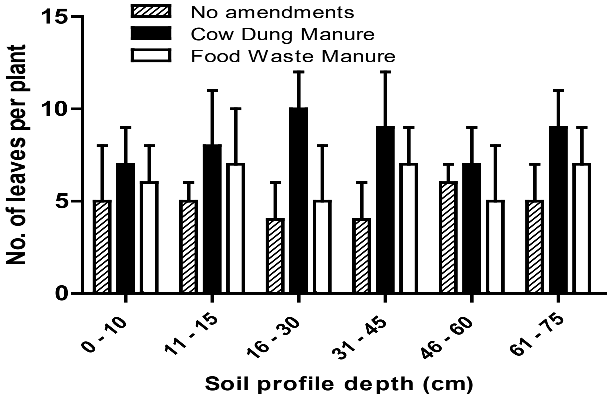


Figure 10. Number of leaves per plant at 5 weeks after sowing and soil amendments.

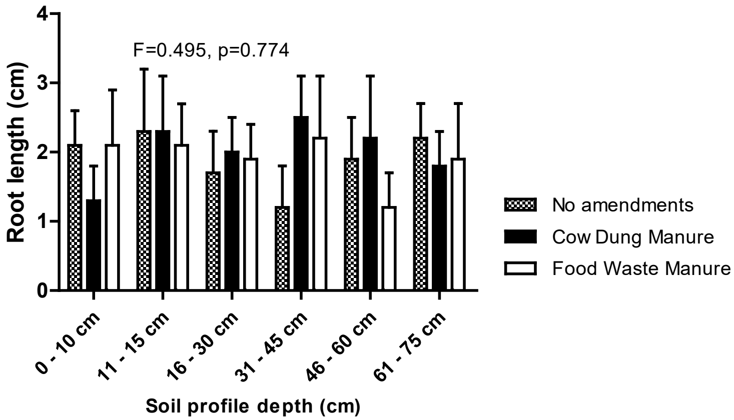


Figure 11. Root length of *Amaranthus viridis* seedlings at 5 weeks after sowing and soil amendments.

Figure 12 presents the total seedling dry weight of *A. viridis* after 5 weeks, originating from 0.05 g seeds. The data reveals significant effects of soil amendment and depth on biomass production. At the 0 - 10 cm horizon, seedlings without soil amendment had a biomass of 0.74 g, which increased to 0.89 g with cow dung amendment. Notably, the mean biomass across all amendment regimens within this horizon was 0.87 g. In contrast, seedlings in the 11 - 15 cm horizon exhibited higher biomass (1.05 g), while those in the deepest horizon (61 - 75 cm) had lower biomass (0.76 g).

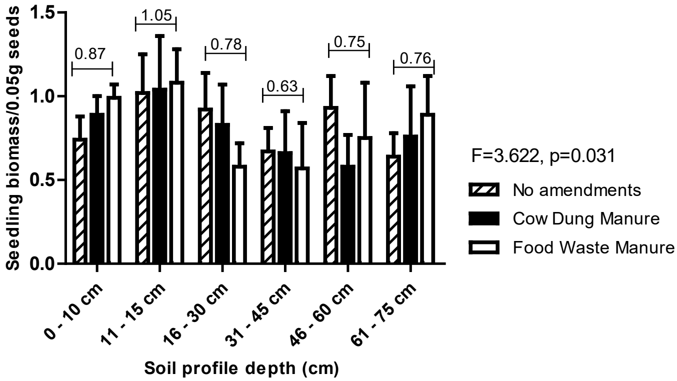


Figure 12. Total seedling biomass

Discussion

This study focused on the impact of soil depth on the growth of *A. viridis* after amendment with organic fertilizer. A primary role of soils is to provide plant roots with the nutrients required for growth and productivity (Miki *et al.*, 2010). Soil is the largest pool of terrestrial organic carbon in the biosphere, storing more carbon than is contained in plants and the atmosphere combined (Schlesinger 1997).

The abundance of organic carbon in the soil affects and is affected by plant production, and its role as a key control of soil fertility and agricultural production has been recognized for more than a century. In agricultural production systems, plant roots grow below 25 cm and the deeper soils are important for crop yield, because top soils may dry out quickly during summer months, limiting the ability of roots to absorb water and nutrients in the upper layers of the soil profile. (Perkons *et al.,* 2014). Since plant growth is dependent on edaphic factors, the plant - soil interactions at different soil depths play a role in the abundance and composition of soil microbial communities. Although most studies have focused on nutrient rich topsoil, the roots of agricultural crops can grow as deep as 200 cm (Perkons *et al.,* 2014).

The subsoil which is also known as the B horizon contains some nutrients thereby making it capable of plant growth. Subsoils range in texture from gravel to heavy clay, in reaction from very acid to strongly alkaline, in fertility from very low to very high, and in structure and consistence from granular and friable to blocky, hard and plastic, or even cemented (Winters and Simonson, 1959; Reuss and Campbell, 1961).

Soils require nitrogen and phosphorus, with manure applications further enhancing response. Microbial abundance and diversity typically decrease with soil depth; this decrease can alter soil properties, while fertilizer incorporation improves crop yields, soil organic matter, and nutrient content (Gao *et al.,* 2015; Choudhary *et al.,* 2018).

Agboola and Unama (1991) emphasized the importance of maintaining soil organic matter in agricultural systems through practices that prevent its destruction or by consistently adding organic materials. Soil contains essential nutrients, and organic fertilizers enhance their availability for increased plant uptake and yield (Ref...). Applying organic fertilizers to the soil surface can provide a rich food source for microorganisms, significantly increasing microbial community composition and diversity (Chang *et al.,* 2007; Diacono and Montemurro, 2010).

Organic wastes such as animal manures, by - products of several kinds and composted residues can be used as amendments to increase soil fertility, since they are important sources of nutrients for growing crops and means for enhancing the overall soil quality (Davies and Lennartsson, 2005). Managing agricultural nutrients to provide a safe food supply and secure the environment remains one of the immense challenges of the 21st century. Crop nutrient uptake and crop yields are the principal factors that determine optimal fertilization practices. Therefore, it is very important to apply fertilizers in an efficient way to minimize loss and to improve the nutrient use efficiency.

Soil organic matter plays an important role in long - term soil conservation and/or restoration by sustaining its fertility, and hence in sustainable agricultural production, due to the improvement of physical, chemical and biological properties of soils (Sequi, 1989). The organic matter content is the result of the inputs by plant, animal and microbial residues, and the rate of decomposition through mineralization of both added and existing organic matter. Fertilization with manure improves soil's physical, chemical, and biological qualities, primarily by decreasing soil bulk density and improving structure (Ullah *et al.,* 2020; Du *et al.,* 2020). Organic fertilizers are applied to soil in precise amounts to increase soil organic carbon (SOC) and other vital plant nutrients, particularly N - P - K and micronutrients (Akhtar *et al.,* 2018; Iqbal *et al.,* 2021).

Soil organic carbon sequestration can be enhanced by fertilization such as incorporation of crop residues or the direct application of manure, which implies by high carbon inputs (Cai *et al.,* 2016). Based on 153 field experiments in China, Chen *et al.* (2014) observed an increase in crop yields of approximately 8.5 - 14.2 Mg ha^{-1} following fertilization with manure without any increase in nitrogen (N) fertilizer. According to literature, food waste cannot be used directly as a fertilizer. When food waste is used as a raw material for fertilization, various problems such as excessive salt content, mixing of impurities and odor arise (Chen *et al.,* 2021).

Conclusions

This study investigated the impact of soil horizons on the growth of *Amaranthus viridis* and the effects of organic fertilizer amendments. The results indicate that deeper soil horizons present less favorable conditions for seedling development. Seedling emergence and overall growth were notably influenced by soil depth, with shallower soil profiles generally supporting better development. The application of organic fertilizers, particularly cow dung manure, enhanced seedling growth and reduced foliar chlorosis. While food waste manure initially hampered seed emergence, it supported growth at later stages. These findings underscore the potential of organic amendments to improve soil conditions and promote *Amaranthus viridis* growth, particularly in less fertile soil horizons.

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