

## Spectroscopic Analysis of the effect of organic and inorganic Fertilizers on the Chlorophylls pigment in Amaranth and Jute Mallow vegetables.

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### Abstract

The effect of six different fertilizers on concentration of photosynthetic pigments in leaves of amaranth (*Amaranthus spp*) and jute mallow (*Corchorus olitorius*) was studied in controlled conditions. Two types of pigments were examined: chlorophyll A and chlorophyll B. The highest concentration of photosynthetic pigments of 18.99 µg/L chlorophyll A; 10.6 µg/L chlorophyll B was found in the leaves of vegetables treated with pig manure and the lowest values of 16.01 µg/L chlorophyll A; 8.33 µg/L chlorophyll B in the vegetables treated with mineral fertilizer NPK 15:15:15 for amaranth, while for jute mallow the highest pigment concentration of 21.74 µg/L chlorophyll A; 12.09 µg/L chlorophyll B was found in leaves of vegetables treated with Urea and the lowest pigments of 19.02 µg/L chlorophyll A; 10.0 µg/L chlorophyll B in the seedlings treated with pig manure. Results show that the concentration of photosynthetic pigments in leaves of amaranth and jute mallow seedlings vary depending on the fertilizer that was applied, as such conducting appropriate fertilizer screening before application is critical in ensuring high yielding vegetables.



## Background

Photosynthetic Pigments are substances with very different chemical structure; they are present in the form of porphyrin pigments (chlorophyll A, B and C), carotenoids, anthocyanins and flavones (Costache *et al.*, 2012). Total leaf pigment includes chlorophyll-A, chlorophyll-B and carotenoids that are necessary for photosynthesis process (Brown *et al.* 1991). The chlorophyll resides in the chloroplasts and gives the green colour of the leaves. This crucial pigment also plays a role as an index of plant growth and production of organic matter (Lahai *et al.* 2003). Chlorophylls and carotenoids are essential pigments of higher plant assimilatory tissues and responsible for variations of color from dark-green to yellow. Moreover, they play important roles in photosynthesis capturing light energy which is converted into chemical energy (Bauernfeind, 1981; Young and Britton, 1993).

Variation in leaf pigments and its relation can be due to internal factors and environmental conditions (Shaikh *et al.*, 2008). The ratio of chlorophyll-a and chlorophyll-b in terrestrial plants has been used as an indicator of response to light shade conditions (Vicas *et al.*, 2010). The small proportion of chlorophyll a or b is considered as sensitive biomarker of pollution and environmental stress (Tripathi and Gautam, 2007). The absorbance properties of pigments facilitate the qualitative and quantitative analysis of them (James and Akaranta, 2011).

In alternative medicine, Chlorophyll has positive effects on inflammation, oxidation, and wound healing. Chlorophyll and chlorophyllin can form complexes structures with certain chemicals causing cancer such as aflatoxin-B1 found in powders and extracts of many spices, herbs and higher plants. The leaves and green parts of plants have been used for centuries to accelerate wound healing. Among the ancients, the greenest plants were chosen for health remedies. In this century, medical scientists have found chlorophyll to be effective in the general fields of detoxification, deodorization, and the healing of wounds.

Chlorophyll concentration usually, is a good indicator of plant nutrient stress, photosynthesis and growing periods, the content of chlorophyll in the plant leaves indicates the growth status of the crops, also it is the important

condition for exchange of mass and energy from the outside world and therefore real-time monitoring of the content of chlorophyll is a key step to complete crop monitoring and yield estimation (Rao *et al.*, 2007; Costache *et al.*, 2012). Also, at the Earth's surface, solar energy is reflected, absorbed or transmitted from all materials (Larcher 2003). In plants, chlorophyll and other leaf pigments are responsible for leaf energy absorption and reflection. Low concentration of chlorophyll results in less energy absorption and more reflectance.

The quantification of chlorophyll provides important information about the effects of various conditions and treatments on plant growth (Schlemmer *et al.*, 2005). Chlorophyll content is an indicator for crop growth and development, therefore accurately determining and assessing of chlorophyll concentration is essential (Bannari *et al.*, 2007).

## Methodology

The study was carried out in open field at Benue State University, Makurdi, Nigeria to determine the effect of inorganic and organic fertilizers on photosynthetic pigments in green-leafy vegetables. The vegetable seeds were sown in polyethene bags filled with loamy soils and irrigated with tap water. The pots were divided into six groups each of which was treated with different kind of fertilizers with control as follows: (a) Control (b) pig droppings (c) cow droppings (d) poultry droppings (e) Chemical fertilizer "Urea" (f) chemical fertilizer superphosphate (SSP) (g) Chemical fertilizer "NPK". Each treatment was performed in three replicates. The pigment parameters were assessed after three weeks of germination.

## Calibration of spectrometer

The Pasco spectrometer was plugged into a computer using the USB cable to pair the spectrometer to a computer running PASCO's Spectrometry Application (<http://pasco.com/spectrometer/>). The spectrometer turned off light sources to perform a dark calibration with the sample covered well with the finger to block any ambient light from entering the detector. A check mark appeared when the calibration was complete. A cuvette was filled 3/4 with 20% ethanol to use for the reference calibration by Placing the cuvette into

the spectrometer so that a clear side is facing the white light source icon. A check mark indicated when the calibration was complete.

### Sample Preparation

Using a balance, 0.50 g of each leaf sample was weighed with the veins and stems avoided by cutting out small leaf tissue sections with a scissors. The samples were placed in a clean mortar and 20 mL of 95 % ethanol added. The mixture was ground with the pestle for 2-3 min until the mixture was homogenized as much as possible. The solution was then filtered using a Whatman no 10 filter paper and funnel into a labelled test tube. A cuvette was filled with the filtered leaf extract before being analyzed with a spectrophotometer (PASCO model: PS-2600).

The absorbance was measured at 663 and 644 wave lengths to determine Chlorophyll A and Chlorophyll B respectively using 95 % ethanol as blank. Then pigment concentrations were calculated in  $\mu\text{g/ml}$  according to the following equations 1 and 2 (Porra *et al.*, 1989):

$$\text{Ch-A } (\mu\text{g/ml}) = 13.36A_{664} - 5.19A_{649} \dots\dots(1)$$

$$\text{Ch-B } (\mu\text{g/ml}) = 27.43A_{649} - 8.12A_{664} \dots\dots(2)$$

**Quality control:** Analytical reagents used during the extraction process were of AR grade (Merck). Distilled water was used for preparation of intermediate solution and for dilution purposes (wherever needed). Glass cuvettes were used and corresponding solvent was taken as reference during spectrophotometric observation. Every procedure (for each plant sample and extracting solvent) was replicated for maintaining the precision of analytical results

### Statistical analysis

All data of the present investigation were subjected to analysis of variance and significant difference among means were determined with the aid of SPSS software. In addition, significant difference among mean were distinguished according to the Duncan's, multiple test range and differences between means were compared at 95% confidence.

### Results and Discussion

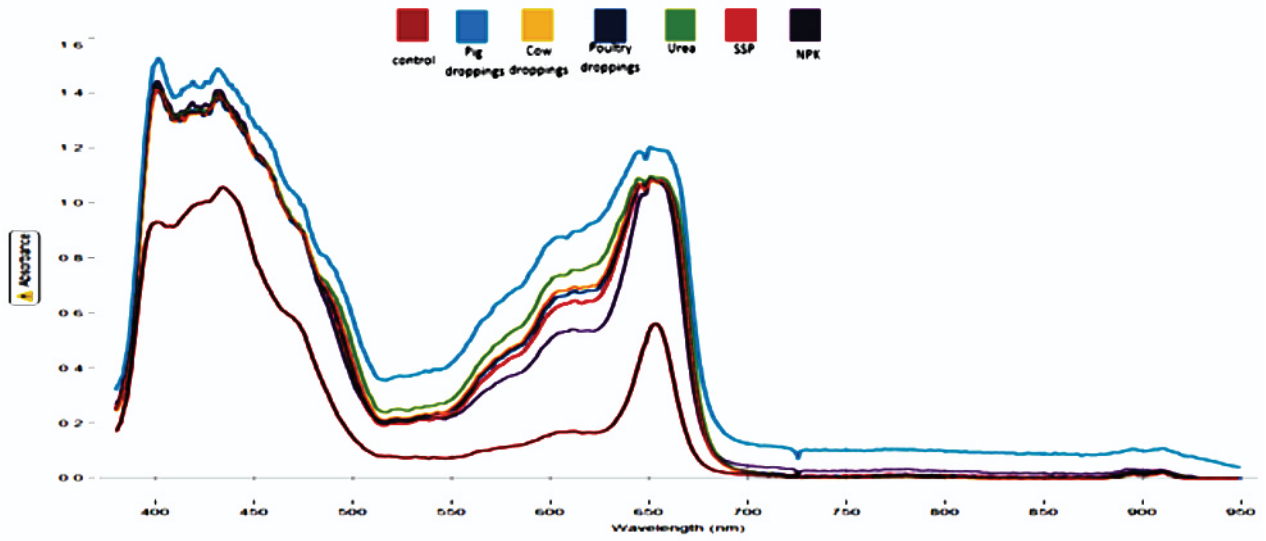
Optical spectrometers usually called spectrometers, displays the intensity of light as a function of wavelength or of frequency. The

different wavelengths of light are separated by refraction or by diffraction. These spectrometers utilize the phenomenon of optical dispersion. The light from a source can consist of a continuous spectrum, an emission spectrum, or an absorption spectrum. Because each element leaves its spectral signature in the pattern of lines observed, a spectral analysis can reveal the composition of the object being analyzed. This method was used to determine the chlorophyll level present in each sample, since the samples absorb light at different intensities using a spectrometer.

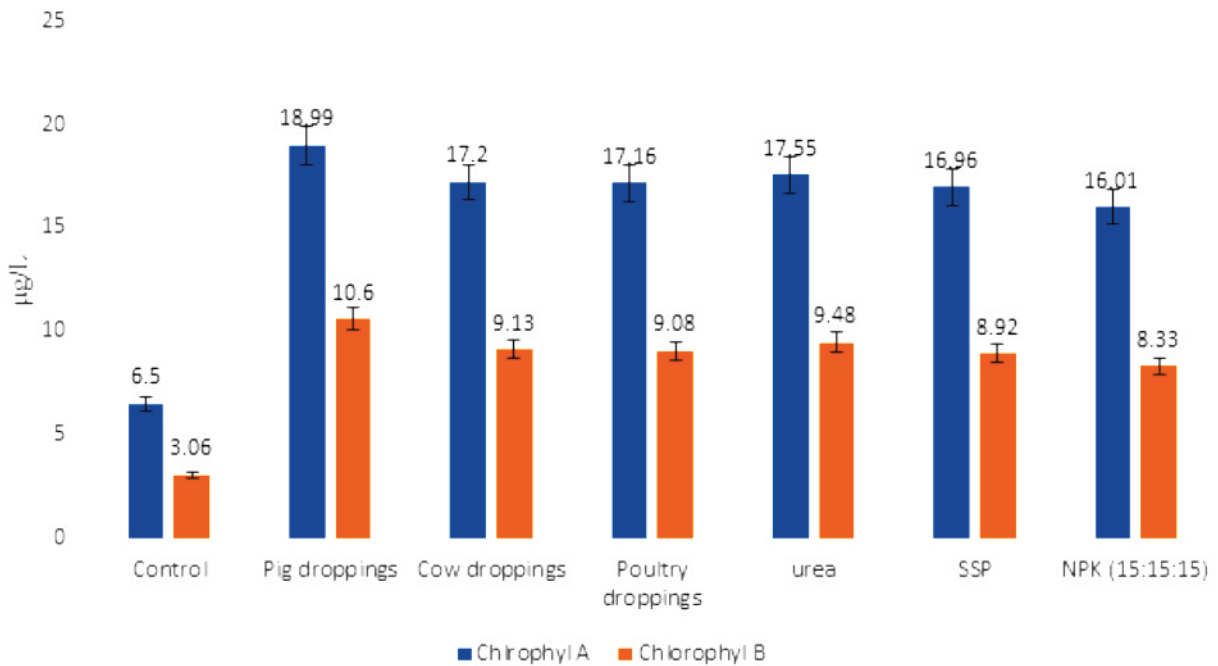
Results presented in Figure 1 and 3 show that there is absorbance at wavelengths of 430 nm, 453 nm, 642 nm and 662 nm which is consistent with chlorophylls A and B. From Figure 2 and 4, it can be observed that there is higher intensity peak signifying higher content of chlorophyll-A in both amaranth and jute leaves as compared to chlorophyll-B. Again, plants that were untreated with fertilizers (control) showed the lowest chlorophyll content in both amaranth and jute leaves. This was similar to reports by El-Arwy *et al.*, (2016) who reported higher chlorophyll-A concentrations and that fertilization enhanced the formation of the pigments and caused an increase in their concentrations either in salt stressed or unstressed plants. Chlorophyll-A is recognized as the main pigments which convert light energy into chemical energy. Chlorophyll-B as accessory pigments acts indirectly in photosynthesis by transferring the light it absorbs to chlorophyll-A (3). In amaranth samples (Figure 2), the plots fertilized with manure produced leaves which contained higher chlorophyll than did leaves from plots using synthetic fertilizers. Pig manure showed significantly the highest chlorophyll content. El-Arwy *et al.*, (2016) similarly reported that as organic fertilizer concentration increased the pigment concentration increased. In their report, treatments with three portions of organic fertilizer caused an increase of about 12.8%, 37.6% and 39.7%, respectively in chlorophyll A; and about 4.6%, 12.9% and 15.7%, respectively in chlorophyll-B. Leaves from synthetic fertilizers revealed that plots high in nitrogen contained more of the chlorophyll pigments than did the leaves from plots from other types of fertilizer. This was different for jute leaves which showed higher chlorophyll

content for plots grown using synthetic fertilizers. NPK fertilized jute leaves showed highest chlorophyll content compared to superphosphate and urea. This is due to the fact

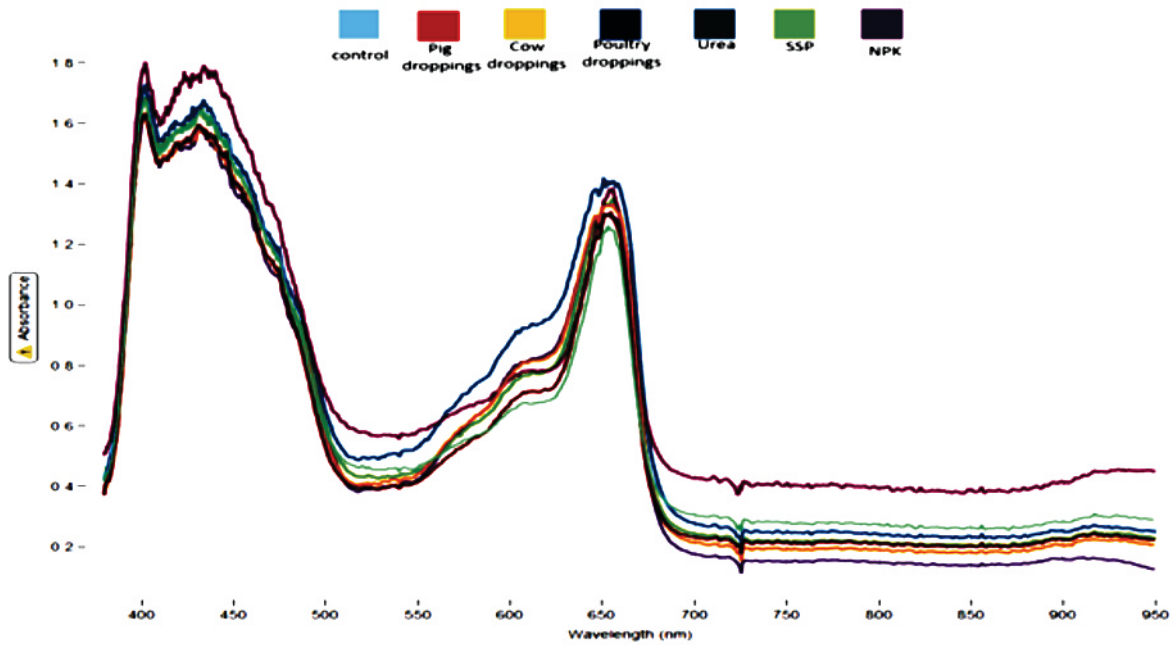
that nitrogen, phosphorus and potassium are the main nutrients required for the growth of most plants. Also, NPK fertilizers are water soluble, allowing easy uptake by the vegetables.



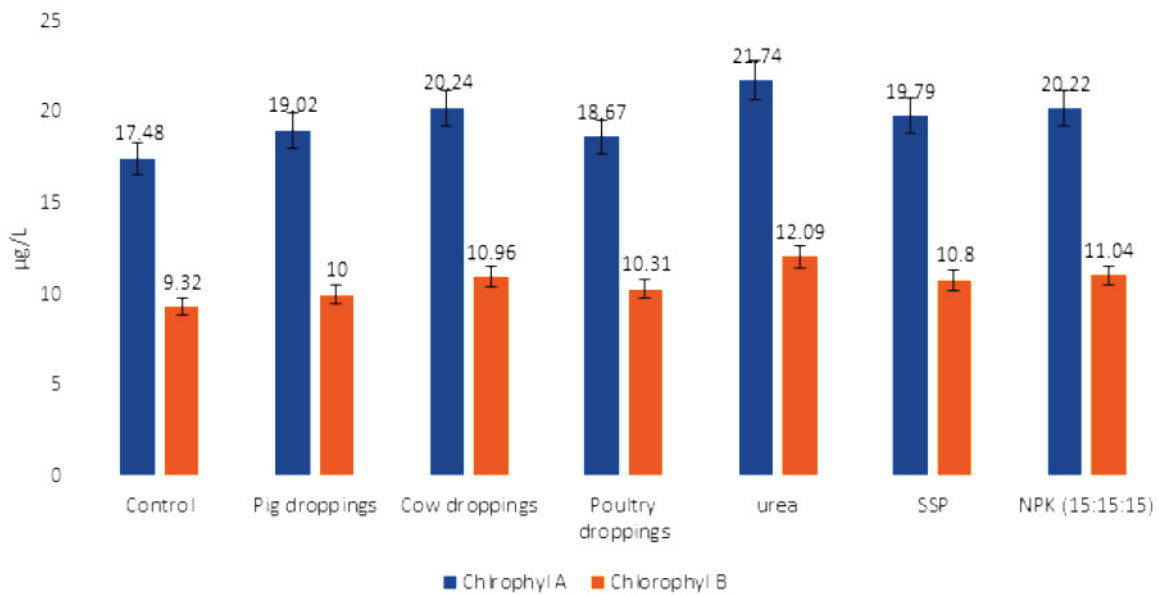
**Figure 1:** Absorbance spectrum of amaranth leaf at various treatments



**Figure 2:** Pigment concentration of Amaranthamaranth leaf at various treatments



**Figure 3:** Absorbance spectrum of Jute mallow leaf at various treatments



**Figure 4:** Pigment concentration of Jute mallow leaf at various treatments

Statistical analysis showed that there was a significant difference ( $p < 0.05$ ) in the pigment concentration for vegetable leaves for different treatments for both amaranth while there was no significant difference ( $P > 0.05$ ) in pigment concentration for jute leaves. This suggests that certain fertilizers do not always produce the same effects upon the development of the chlorophyll pigments for different plants; and the effect is apparently due to some factors which the fertilizer influences indirectly. This is most likely seen in the variation in pattern and effect of the fertilizers on different plants.

Plant pigments serve as antioxidants, and can be a source for vitamin A activity

(Britton *et al.*, 1995). A number of the studies on cancer preventative effects of chlorophyll derivatives have been done (Egner *et al.*, 2003; Ching-yun *et al.*, 2008). Chlorophyll is a good source of antioxidant nutrients. Antioxidant nutrients such as vitamins A, C and E help to neutralize harmful molecules (free radicals) in the body that can cause damage to healthy cells. Many studies support that chlorophylls and its derivatives have antioxidant properties (Feruzzi *et al.*, 2002). The chlorophyll molecule has  $Mg^{2+}$  at its center which makes it ionic and hydrophilic, and a ring that is hydrophobic in nature with a carbonyl group at its tail which makes it polar. It is held in place in the plant cell

within a water-soluble chlorophyll-binding protein (WSCP). Chlorophyll-B differ from chlorophyll-A only in one functional group (i.e -CHO) bounded to the porphyrin ring, and is more soluble than chlorophyll-A in polar solvents because of its carbonyl group (Lichtenthaler and Wellburn).

No significant differences were observed in the trend for pigment extraction as reported in other works on phytoplankton and also from higher plants with the present study. Though slight variation has been reported to persist among experimented plants and species even for same extractant solvent which can be attributed to inherent physiological characteristics of individual species. Temporal and seasonal changes and local geological condition can also be the reason for variations in pigment concentrations in plants.

### Conclusion

The Results obtained during the analysis of cultivated vegetable crops in the field indicate that the extraction of photosynthetic pigments by 95 % ethanol used as solvent depends upon the chemical nature of bio-molecules or photosynthetic pigment molecules. Results showed high content of chlorophyll-A and Chlorophyll-B in the treated plants in comparison with the control plants. The content of chlorophyll are as follows: pig manure > urea > poultry droppings > cow droppings > SSP > NPK > control for amaranth leaves while for jute mallow it was urea > NPK > pig dropping > cow droppings > SSP > poultry droppings > control. By the application of recommended organic and inorganic fertilizers to the vegetables, differences were observed in the concentrations of pigments between treated and control plants selected for present study. Though variations may persist among the experimented fodder crops due to physiological and environmental conditions, local geological conditions may also cause variations in pigment concentrations. However, further analysis is recommended in this context.

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