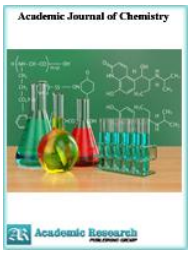

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Effect of Seasonal Water Fluctuation of a Water Body on Antioxidant Activity of Selected Plants of Lower Phylum (A Case Study of Nche stream)

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Abstract: Effect of seasonal water fluctuation of a water body on antioxidant activity of selected plants of lower phylum using Nche stream as a case study was investigated using standard methods. Three plants of lower phylum (watercress, moss plant, and spirogyra) were selected and studied for both enzymatic and non-enzymatic antioxidants. Results obtained for levels of ascorbic acid (0.81-11.87 µmoles/g DW), glutathione (1.47-3.01µmoles/g DW) and proline (1.27-3.01 g/100g) non-enzymatic antioxidants and those of superoxide dismutase (289.19-615.85 µg/g protein), peroxidase (32.56-52.79 µg/g protein), and catalase (57.80-73.20µmoles/g DW) of enzymatic antioxidants were higher in dry season against rainy season. It has been noted that a slit difference in these indicators could be as result of enormous stress. The reduction in volume of water of the host stream in dry season may have resulted in increased concentration of the pollutants in the water body hence, inducing the plants to absorb more of the pollutants. This may have triggered more stress on the plants, which reflected on the levels of the observed stress indicators when compared to the indicators as observed in rainy season. This study has shown the seasonal water fluctuation of a water body on antioxidant activity of selected plants of lower phylum.

Keywords: Antioxidant; Lower phylum; Seasonal fluctuation; Stress enzymes; Water body.

1. Introduction

According to Dominika and Barbara [1], biological systems stress can be defined as an adverse force, effect, or influence that trends to inhibit normal systems from functioning. It has been noted that a wide range of unfavourable environmental conditions may induce stresses in plants, which can alter their growth, development, metabolism, and even may lead to death Dominika and Barbara [1]. Plants react to environmental stresses on various levels including biochemical, cellular and morphological scales depending on type of species or population [2-5]. Abiotic stress is defined as the negative impact of non-living factors on living organisms in a specific environment [6]. Basically, reaction of plants to abiotic stresses depends on type of plant species due to fundamental differences in development and anatomy as well as environmental limiting factors [7].

Water stress is among the abiotic stresses that affect plants [2]. This type of stress could be generated by drought or flooding [2]. According to Bartels and Souer [8], water deficit, caused by "lack of water" has been among the problems for agriculture, affecting virtually every aspect of plant physiology and metabolism. The mechanisms of abiotic stress effect on plants have been reported by different authors [9-11], and have been linked to generation of reactive oxygen species (ROS), which include O_2^-, H_2O_2, and OH^-. These molecules are highly reactive and can alert normal cellular metabolism through oxidative damage to membranes, proteins and nucleic acids [10]. Reactive oxygen species can also cause lipid peroxidation, protein denaturation and DNA mutation [12].

Generally, to prevent the damage inherent from reactive oxygen species (ROS) on cellular components, plants have developed a complex antioxidant system[10]. According to Rahimizadeh, *et al.* [10], the primary components of this system include carotenoids, ascorbate, glutathione and tocopherols. Others are superoxide dismutase (SOD;

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EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), perioxidase (POX; EC 1.11.1.7) and glutathione reductase (GR; EC 1.1.4.2) [12]. The antioxidant enzymes play key role in defense against oxidative damage [9-11].

Nche stream is among the natural water bodies that supply domestic water to the people of Umunchi. Umunchi is among the communities found in Isiala Mbano L.G.A of Imo State, Nigeria. Water level of the stream is known to increase in rainy season, and decrease during dry season. Apart from domestic water supply, the stream houses other organisms that depend on it. An earlier study on the water by Duru, *et al.* [13], ascertained the seasonal water quality assessment of the water body. There is need to extend the study on the water body to accommodate lower organisms that depend on water from the water body.

Different studies have investigated the effect of water stress on plants, but most of the studies were centered on drought stress [14-19] through isolated observations. The present study investigated the seasonal water fluctuation of a water body on antioxidant activity of selected plants of lower phylum, with a view to ascertain the stress induced by such fluctuation, using Nche steam as a case study.

2. Materials and Methods

2.1. Location of Nche Stream

Isiala Mbano L.G.A lies within latitude 5°40´ 3.6´´ (5.6677°) north and longitude 7 ° 12´ 22.2´´ (7.2034°) east with an average elevation of 149 meters (about 489 feet). Within these latitude and longitude lies Umunchi community, and hence Nche stream.

2.2. Collection and Preparation of Samples

Three plants of lower phylum were used for this study. The plants were spirogyra (seaweed), watercress (*Nasturtium sp*.) and mosses. Spirogyra (seaweed) sampling was done using the method described by Okafor [20] for phytoplankton. Coned-shaped, silk plankton net was employed. At each free-flowing part of the water body, a net was used by sinking and drawing it against the water current. Those found on hard surfaces such as the walls built by local population to safe-guide the stream were collected with the help of sterile scraper. Watercress (*Nasturtium sp*.) samples were collected from banks of the stream. Moss plants used in this study were collected from the walls as built by the local population to protect the stream, making sure that they receive water from the stream both at high tide and low tide. The samples were transported to the laboratory in a cold condition (container packed with ice). At the laboratory, the sampled plants were prepared for further studies. The sampling was done at peak of the two seasons.

3. Determination of Non-Enzymatic Antioxidants

The titrimetric method described by Conklin [21] was used to determined ascorbic acid. Glutathione and phenol were determined as described by Muruganand and Harish [22]. Carotene was determined with the method as described by Dere, *et al.* [23]. Speckman, *et al.* [24] method was used for proline while flavonoid was determined using AOAC [25] method.

4. Estimation of Enzymatic Antioxidants

The method as described by Lowry, *et al.* [26] was used for protein assays. Measurement of superoxide dismutase enzyme activity was done using the method of Misra and Fridorich [27]. Peroxidase enzyme activity was estimated using the method as described by AOAC [25]. Catalase activity was measured using Paglia and Valentine [28] method. Activity of Glutathione reductase (GR) was estimated using the method of Foyer and Halliwell [29], as modified by Rao [30].

5. Results and Discussion

Fig-1. Ascorbic acid levels of the plants.

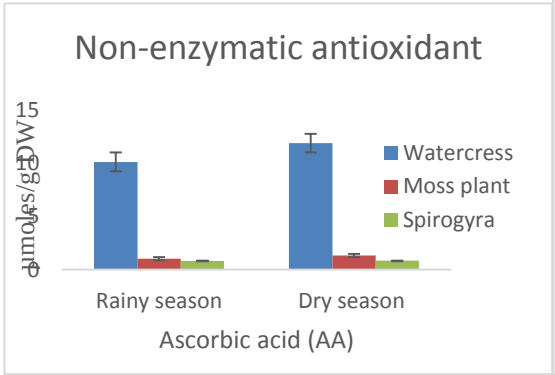


Fig-2. Glutathione levels of the plants.

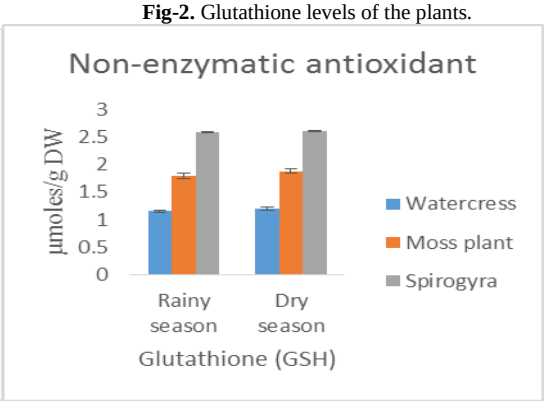


Fig-3. Phenol levels of the plants.

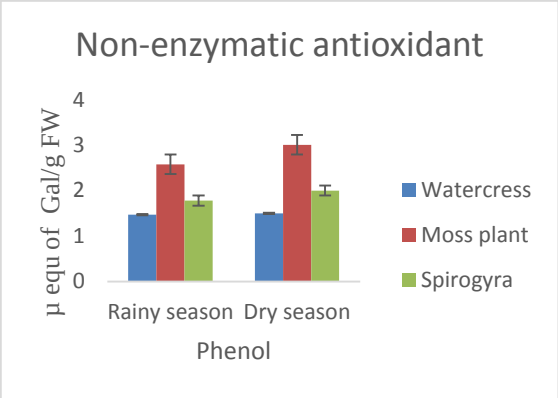


Fig-4. Carotene levels of the plants.

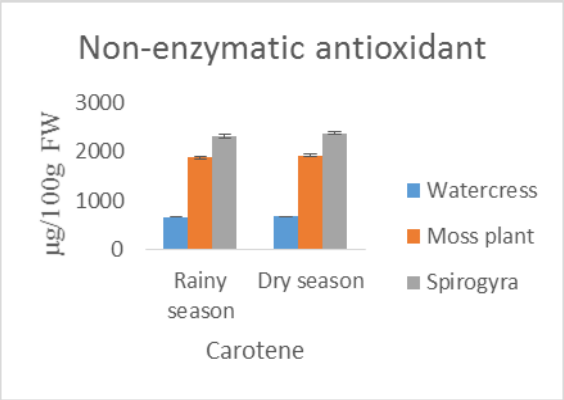


Fig-5. Flavonoid levels of the plants.

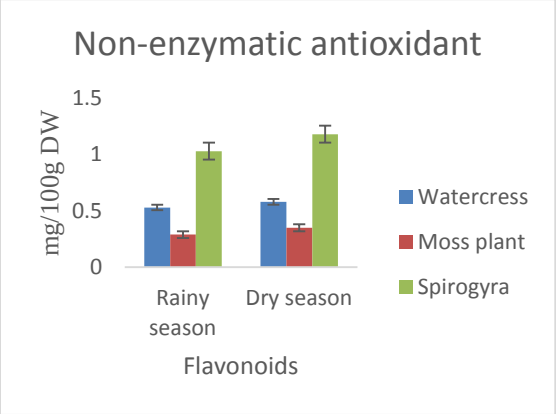
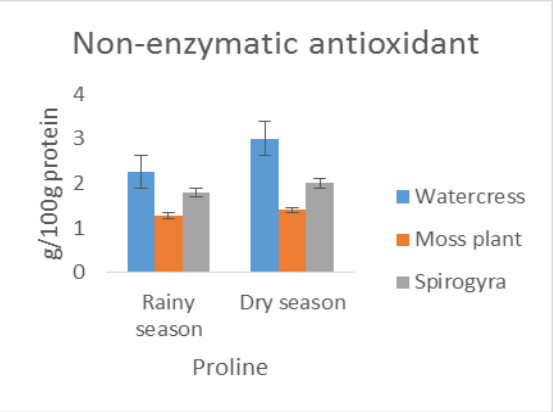


Fig-6. Proline levels of the plants.



Figures 1-6 show the presence of non-enzymatic antioxidants found in the studied plants. Ascorbic acid, also known as vitamin C is the most abundant, powerful and water soluble antioxidant which minimizes or prevents damage caused by reactive oxygen species (ROS) in plants. Ascorbic acid (AA) is one of the most studied and has been detected in majority of plant cell types, organelles and appoplast [31]. Wu, *et al.* [32] noted that ascorbic acid reacts not only with H_2O_2, but also with O_2^-, OH and lipid hydroperoxidases. Ascorbic acid can also directly scavenge $1O_2$, $O_2^-$and HO. and regenerate tocopherol from tocopheroxyl radicals providing membrane protection [1]. Smirnoff and Wheeler [31] summarized that ascorbic acid reacts non-enzymatically with superoxide, hydrogen peroxide and singlet oxygen. Ascorbic acid levels of the studied plants were between 10.12-11.87 µmoles/g DW in watercress; 1.02-1.32 µmoles/g DW in moss plant and 0.81-0.84 µmoles/g DW in spirogyra (Figure 1). Glutathione (GSH) is a tripeptide (α-glutamyl-cysteinyl-glycine), which is considered as the most important intracellular defense against ROS-induced oxidative damage [1]. Glutathione is important in plant chloroplasts because it helps to protect the photosynthetic apparatus from oxidative damage [33]. Glutathione levels of the present study ranged from 1.15-1.20 µmoles/g DW in watercress; 1.79-1.88 µmoles/g DW moss plant; and 2.58-2.60 µmoles/g DW in spirogyra (Figure 2). It has been noted that phenols are aromatic secondary metabolites broadly distributed in plant kingdom [34]. They are essential to physiology and cellular metabolism [35]. Phenolic compounds play a role of protection against insects and other animals to the plants. The antioxidant activity of phenolic compounds depends largely on their chemical structures. Among the phenolic compounds with known antioxidant activity are flavonoids, tannins chalcones and coumarins as well as phenolic acids [35, 36]. According to Dai and Mumper [37], phenolics have been considered as great antioxidants and proved to be more effective than Vitamin C, E and carotenoids in recent times. Phenolic levels of the present study were between 1.47-1.58 µ equ. of Gal/g in watercress; 2.58-3.01 µ equ. of Gal/g in moss plants; and 1.79-1 88 µ equ. of Gal/g in spirogyra (Figure 3). Carotenoids are lipid soluble antioxidants pigments that play multitude of function in plant metabolism including oxidative stress tolerance [1]. Carotenoids observed in the present study were between 660.10 -671.60 µg/100g FW in watercress; 1870.13 to 1920.24 µg/100g FW in moss plant; and 2310.00-2370.15 µg/100g FW in spirogyra as presented in Figure 4. Flavonoids are widely distributed in plants; and come in four classes depending on their structure; flavonols, flavones, isoflavones and anthocyanines [1]. Flavonoids belong to one of the most reactive secondary metabolites of plants [37], and play important role as reactive oxygen species (ROS) scavenger by locating and neutralizing radicals before they damage cell structure Dominika and Barbara [1]. It has been proved that they are involved in plant responses to both, biotic or abiotic stresses such as wounding, drought and metal toxicity [38]. Flavonoids level of the present study ranged from 0.53-0.58 mg/100g in watercress; 0.29-0.35 mg/100g in moss plant; and 1.03-1.18 inspirogyra (Figure 5). Proline as α-amino acid, is an antioxidant and potential inhibitor of programmed cell death[1]. It has been suggested that free proline acts as osmoprotectant, a protein stabilizer, a metal chelator, an

inhibitor of lipid peroxidation and OH· and $1O_2$ scavenger [1]. Proline is not only an important signaling molecule, but also an effective reactive oxygen species (ROS) quencher [1, 39]. Levels of proline in the present study were between 2.26-3.01 g/100g protein in watercress; 1.27-1.40 in moss plant; and 1.79-1.85 inspirogyra (Figure 6). Trovato, *et al.* [40] reported increased proline accumulation during abiotic stresses. It has been found that the important role of proline is in potentiating pentose-phosphate pathway activity as important component of antioxidative defense mechanism [39]. Flavonoids are widely distributed in plants leaves, floral part and pollens. They often accumulate in the plant vacuole as glycosides or as exudates on the leaves surface and other aerial part of the plant. There are four flavonoid classes depending on their structure: flavonols, flavones, isoflavones and anthocyanines. Flavonoids belong to one of the most reactive secondary metabolites of plants [41]. Flavonoids play important role as ROS scavenger by locating and neutralizing radicals before they damage cell structure. Flavonoids have function as flowers, fruits and seed pigmentation, they play protective role before UV light, drought, cold, and defense against pathogens. Flavonoids play an important role in plant fertility and germination of pollen. They are involved in plant signaling with interaction with plant microbes [41, 42]. It has been proved that they are involved in plant responses to both, biotic or abiotic stresses such as wounding, drought and metal toxicity [38]. Proline**,** α-amino acid is an antioxidant and potential inhibitor of programmed cell death. It has been suggested that free proline act as osmoprotectant, a protein stabilizer, a metal chelator, an inhibitor of lipid peroxidation and OH· and $1O_2$ scavenger. Increased proline accumulation appears especially during salt, drought and metal stresses [40]. Therefore proline is not only an important signaling molecule, but also an effective ROS quencher. It has been found that the important role of proline is in potentiating pentose-phosphatase pathway activity as important component of antioxidative defense mechanism [39].

Fig-7. Superoxide dismutase levels of the plants.

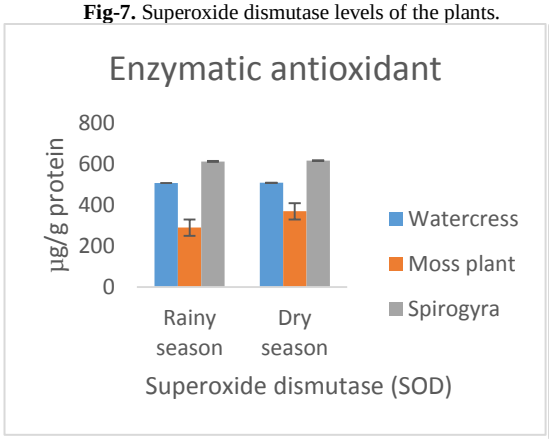


Fig-8. Peroxidase levels of the plants.

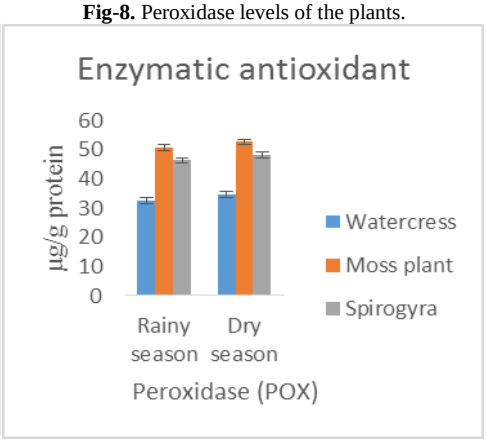


Fig-9. Catalase levels of the plants.

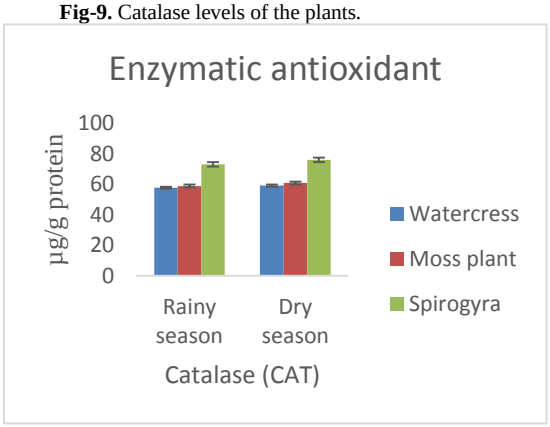
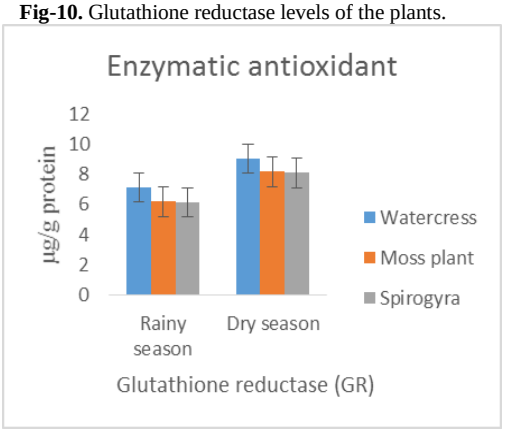


Fig-10. Glutathione reductase levels of the plants.



According to Dominika and Barbara [1], superoxide dismutase (SOD) constitutes the first line of defense against reactive oxygen species (ROS). SODs are metalloproteins, and based on their metal cofactor, they are classified into three known types; the copper/zinc (Cu/Zn-SOD), the manganese (Mn-SOD) and the iron (Fe-SOD) that are localized in different cellular compartment [43]. Dominika and Barbara [1] noted that SODs remove O_2^- by catalyzing its dismutation, one O_2^- is reduced to H_2O_2 and another to O_2. Superoxide dismutase of the present study ranged from 506.94-508.10 µg/g protein in watercress; 289.19-369.02 µg/g protein in moss plant; and 612.09-615.85 µg/g protein in spirogyra (Figure 7). Peroxidase could be in the form of glutathione peroxidase, ascorbate peroxidase or guaiacol peroxidase [1]. According to Abdollah, *et al.* [11], the activity of peroxidases depends on plant species and stress condition. Peroxidase levels of the studied plants ranged from 32.56-34.67 µg/g protein in watercress; 50.89-60.87 µg/g protein in moss plant; and 46.38-48.29 µg/g protein in spirogyra (Figure 8). Catalase is a light-sensitive protein that has a high rate of turnover and environmental stresses which reduce the rate of protein turnover, such as salinity, heat shock or cold, cause the depletion of catalase activity [44, 45]. Dominika and

Barbara [1] noted that it remains unclear whether variability in catalase response to different unfavourable conditions may be of importance in plant stress tolerance level. Observed catalase levels as presented in Figure 9, ranged from 57.80-59.21 µg/g protein in watercress; 58.90-60.87 µg/g protein in moss plant; and 73.20-76.14 µg/g protein in spirogyra. Glutathione reductase is a flavor-protein oxidoreductase that is thought to play an essential role in defense system against reactive oxygen species [42, 46]. Glutathione reductase levels of the present study ranged from 7.14-9.04 µg/g protein in watercress; 6.20-8.18 µg/g protein in moss plant; and 6.16- 8.10 µg/g protein in spirogyra.

All the antioxidants both the non-enzymatic and enzymatic of the investigated plants, increased in dry season than rainy season.

6. Conclusion

Since, it has been noted that a slit difference in antioxidant levels could be as a result of enormous stress. It therefore follows that the observed increase in antioxidant levels of the investigated plants in dry season could be as a result of increased concentration of pollutants found in the host stream due to reduction in volume (low tide) of the water body in dry season. The plants may have absorbed more of the pollutants in dry season, which may have stressed the plants hence, leading to increased levels of the antioxidants in dry season against those of the rainy season where dilution of the pollutants due to increase in level of water of the water body, and their reduced absorption, may have induced a reduced stress on the plants. This study has shown the effect of seasonal water fluctuation of a water body on antioxidant activity of selected plants of lower phylum.

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