Western Ghats terrestrial microalgae serve as a source of amylase and antioxidants enzymes

Manoj BS, Sushma, Mohan Chavan and Arti Karosiya

Abstract
The purpose of the study was to screen six identified microalgae samples from in and around of the Western Ghats Karnataka state for their amylase and antioxidants activity assay. These six collected terrestrial microalgae were grown in plastic tray for multiplication purpose treated with R.O. residual dispense water, pH 8.4 (Ti) and urban waste water (T2) which reduced the pH and TDS content in that water. Rhizoclonium hieroglyphicum has greater chlorophyll content, which helps in multiplication of microalgae very quickly. Carbohydrate degrading enzymes were analyzed using DNS reagents. Free radical scavenging activities were also determined using DPPH (2, 2-diphenyl-1-picrylhydrazyl) method. Superoxide Dismutase and Peroxidase was the most important antioxidant enzymes that protect against the peroxidation system and maintain the redox state of the cell. Amylase, SOD and POD activities were confirmed by conducting native PAGE.

Keywords: antioxidants, DPPH, free radical, Rhizoclonium hieroglyphicum, superoxide dismutase, terrestrial microalgae

Introduction
Microalgae were very effective in reduction of BOD, COD, NH₃, NO₃, PO₄ and TDS in sewage waste water. Microalgal strains generate oxidative stress triggered by reactive oxygen species (ROS) leading to degradation of cellular macromolecules [5]. All tested microalgae extracts possessed the ability to scavenging DPPH at various degrees. SOD, CAT and POD are important antioxidant enzymes in the algal cell that protect against the peroxidation system and maintain the redox state of the cell [7]. Microalgae contains wide range of biochemical compounds and antioxidant agents; Radical scavenging activity of antioxidants determined by using DPPH [10]. Environmental or artificial stress affects the growth, photosynthesis or other physiological processes by the increase in the production of ROS in cells. SODs catalyze the disproportionation of two molecules of superoxide radical ion to oxygen molecule and hydrogen peroxide molecule and it further scavenged by catalases and peroxidases [15]. Production of algal oil is not only for biofuel but also for high nutritional values of oils. The increase in total lipids in algal cells under various stress conditions consisted primarily of neutral lipids, it’s because of the shift in lipid metabolism from membrane lipid synthesis to the storage of neutral lipids [16]. The amylyolytic activity of the enzyme extracts was determined using starch as a substrate by the saccharification method, which is based on production of reducing sugars [17]. The Phycoremediation is an alternative methodology for treating waste water at cheaper cost [20, 22]. The powerful antioxidants minimize oxidative damage to living cells and prevent oxidative deterioration [26]. In present study evaluated the activity of antioxidative enzymes such as SOD and POX on different terrestrial microalgae.

Materials and Methods
The experiments were carried out in the laboratory of Agriculture Biotechnology (Agriculture College Hassan), University of Agricultural Sciences, Bengaluru, Karnataka and also in the laboratory of Biochemistry and Biochemical Engineering, JIBB, SHUATS, Allahabad, U.P. India.

Collection of terrestrial microalgae
Terrestrial microalgal samples were collected in autoclaved plastic bags from water bodies like ponds and rivers of Hassan, Kodagu and Udupi district of Western Ghats of Karnataka. Six identified terrestrial microalgae collected from rivers and ponds of fresh water bodies used for analysis includes Oedogonium sp., Rhizoclonium sp. and Spirogyra collected from the pond of Gadanalhalli, Dandiganahalli and Hoovnalli villages, respectively of Hassan district; Klebsormidium sp. and Rhizoclonium hieroglyphicum from the rivers of Ikola and Kaggodu.
villages of Kodagu district and *Rhizoclonium* sp. collected from the river of Happanadka village of Udupi district.

**Multiplication of terrestrial microalgae in plastic tray**

Collected terrestrial microalgae were kept for multiplication under direct sunlight in plastic tray using normal tap water (Fig. 1). Evaporation was controlled by kept the algae cultured plastic tray under 50% shade net and maintaining the water level by adding extra water for every 5 days interval.

**Effects of terrestrial microalgae on waste water pH and TDS**

The pH and TDS was tested by using the portable pH and TDS meter, respectively. During the time of analysis, taken 10ml of water sample from the small plastic trays treated with different water and terrestrial microalgae and noted down the digital value showed in the pH meter and TDS meter (ppm.)

![A] Rhizoclonium sp. (Happanadka), [B] Rhizoclonium sp. (Dandiganahalli), [C] Spirogyra sp. (Hoovnalli), [D] Klebsormidium sp. (Ikola), [E] Rhizoclonium hieroglyphicum (Kaggodu) and [F] Oedogonium sp. (Gadanahalli)

**Fig 1: Multiplication of terrestrial microalgae in plastic tray.**

**Chlorophylls estimation from terrestrial microalgae**

The chlorophyll is the essential component for photosynthesis, and occurs in chloroplasts as green pigment in all photosynthetic plant tissues. There are various types of Chlorophyll, Chlorophylls a and b occurs in higher plants, ferns and mosses. Chlorophylls c, d and e are only found in algae and in certain bacteria. Chlorophyll (a, band total) estimation was done by Sadasivam and Manickam (1992) methods. For extraction of chlorophyll ground 1g sample using liquid nitrogen and made fine pulp with 20ml 80% acetone. Centrifuged the sample at 6000 rpm for 10 min by adding extra water for every 5 days interval. Repeated the same process un

**Assay for amylase enzyme in terrestrial microalgae**

Amylase was extracted by grinding 0.5 gm of the acetone defatted algal sample with 2.5 ml of 0.1M phosphate buffer (pH 7.4) and at 4 °C centrifuged the sample for 10 min at 6000 rpm. Added equal amount of chilled acetone to supernatant and kept at -20 °C. After 30min, centrifuged at 12000 rpm for 15 min, supernatant was discarded and pellet was dissolved in 1 ml phosphate buffer (0.1M, pH 7.4) and used for amylase estimation. Pipetted out 1 ml starch solution and 1ml of properly diluted enzyme (1:5) (extracted sample) in a test tube, incubated at 27 °C for 15 min and the reaction stopped by adding 2 ml of DNS reagent. Heated solution in a boiling water bath until color developed i.e. 8 min. While the tubes were warm, added the 1 ml of potassium sodium tartrate solution, cooled it and made the volume to 10 ml by adding 6 ml of distilled water and measured the absorbance at 560 nm using spectrophotometer. Standard graph with 200-1200 µg maltose was prepared [8, 14]. Amylase activities was calculated by below formulae

\[
\text{Absorbance at 560 nm} = \text{Final volume including DNS} \\
\text{Time of hydrolysis} \times \text{Extinction coefficient} \times \text{Enzyme used (ml)} \times \text{cuvette diameter (cm)}
\]

Native PAGE was done by using Electrophoresis (Life technologies mini gel tank), which contains 10% resolving gel and 5% stacking gel (Table 1). Run the gel by connecting the cathode and anode to the power bank and set the 60 v, 450 Amp for 2 hrs [19]. Washed the gel in distilled water and incubated in a mixture containing soluble starch (2%) and sodium acetate buffer (0.1M, pH 5.0) at room temperature for 2 hrs. Once again washed the gel in distilled water to remove excess starch solution and kept the gel in a container for 2hr in distilled water. Gel was stained by HCl (0.2 M) containing I$_2$ (5.7 mM) and KI (43.3 mM). The activity band was observed after 10 min of incubation with stain. The whitish band under violet blue background was observed after washing the stained gel in distilled water 2-4 times [9].

Table 1: Native - PAGE recipes for antioxidant enzymes

<table>
<thead>
<tr>
<th>Particles</th>
<th>Gel Resolving (30 ml)</th>
<th>Stacking Gel (10 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>11.9 ml</td>
<td>3.4 ml</td>
</tr>
<tr>
<td>30% acrylamide mix</td>
<td>10 ml</td>
<td>0.83 ml</td>
</tr>
<tr>
<td>Tris buffer (1.5M, pH8.8)</td>
<td>7.5 ml</td>
<td>-</td>
</tr>
<tr>
<td>Tris buffer pH(1.0M, 6.8)</td>
<td>-</td>
<td>0.63 ml</td>
</tr>
<tr>
<td>10%-APS</td>
<td>0.3 ml</td>
<td>0.05 ml</td>
</tr>
<tr>
<td>TEMED</td>
<td>0.012 ml</td>
<td>0.005 ml</td>
</tr>
</tbody>
</table>

Assay for antioxidants activity in terrestrial microalgae

**DPPH (2, 2-diphenyl-1-picrylhydrazyl) activity**

Ground 0.2 gm of the acetone defatted algal sample with 2 ml of 0.1M acetone in a pestle and mortar and ground properly at 4 °C. Incubated the sample for 1 hour at room temperature, shakes occasionally and centrifuged it at 6000 rpm for 10 min at 4 °C. Collected supernatant used as enzyme. For estimation...
taken 0.2 ml of aliquot sample in a 2 ml glass vial covered with aluminum foil, which prevent the direct expose of bright light. Added 80 µl of 7.4 pH Tris-HCl buffer and mixed well. Then added 100 µl DPPH to the reaction mixture and made the volume to 1ml by adding remaining water. Mixed vigorously in dark for 20 minute and measured the absorbance at 517 nm. [23]. Mixture of acetone, Tris-HCl and water was used as blank. DPPH along with acetone, Tris-HCl and water treated as control. Radical scavenging activity by DPPH method was calculated by using the formulae given below:

\[
\text{DPPH activity} = 1 - \frac{\text{Absorption reading of sample}}{\text{Absorption reading of control}} \times 100
\]

**Peroxidase activity**

Guaiacol + H₂O₂ → DPD Oxidized Guaiacol + 2H₂O₂

Ground 0.5 gm of the acetone defatted algal sample with 2.5ml of phosphate buffer (0.1M, pH 7.1) at 4°C and centrifuged for 15 min at 6000 rpm by maintaining 4°C. Collected the supernatant and used as enzyme. Prepared the solution A and solution B reagent mixtures separately. Solution A contains combination of 1.5 ml of phosphate buffer solution (pH 6.1), 0.5 ml Guaiacol solution, 0.1 ml of enzyme and make up 3 ml reaction mixture by adding distilled water. Solution B contains all reaction mixture of Solution A along with 0.5 ml H₂O₂. Solution A containing cuvette was kept in reference place and used as blank. Solution B showed the peroxidase activity at 470 nm in spectrophotometer (CECIL CE 7400), set for enzyme kinetics to show reading every 10 s up to 120 s. Peroxidase activity was calculated based on 30 s difference between any two values [7]. Native PAGE was also carried out based on Sambrook and Russell (2001). PAGE gel was stained by incubating the gel inside the staining mixture containing 100 µl of NBT (0.1%), 500 µl of EDTA (0.2 M), 50 µl of phosphate buffer (100 mM, pH 7.4) and 330 µl of TEMED; Solution B as 30 µl of riboflavin (0.005%). Staining mixture was prepared by adding both solution A and Solution B simultaneously in plastic gel tray. Incubate the gel inside the plastic gel tray containing combination of staining mixture for 15 min in dark without expose to direct light. After 15 min gel was exposed to the light for 3-5 min. Whitish coloured bands were observed with dark bluish background.

**Results and Discussion**

Highest decreasing percentage of TDS was observed in *Rhizoclonium sp.* (Dandiganahalli) and *Rhizoclonium hieroglyphicum*. It is because of the absorbing salt contents in the treated water and used as nutrients for their multiplication of cell and yielding good quantity of biomass. 50th day change in percentage of decreasing TDS content was observed in currently studied terrestrial microalgae are 8.34, 41.76, 14.97, 12.11, 38.70 and 16.93%, respectively on R.O. residual dispense water whose pH 8.4 (T1) and 8.36, 28, 12.27, 10.85, 28.07 and 8.94%, respectively on urban waste water (T2). The pH content in the water T1 and T2 was gradually decreasing after 10th days in all present studied (Table 2). These results indicates that all selected species are converting the alkaline water into acidic. And it was the clear indication that the selected species are well adapted to acidic water of pH 5.49 - 6.4.

**Superoxide Dismutase**

Superoxide Dismutase is a metal containing enzyme with a vital role in scavenging superoxide (O₂⁻) radicals.

\[
O₂⁻ + 2H⁺ → H₂O₂ + O₂
\]

So hydrogen peroxide is eliminated by peroxidases and catalases. Ground 0.5 gm of the acetone defatted algal sample with 2.5 ml of 0.1M phosphate buffer in a pestle and mortar. Centrifuged at 6000 rpm for 15 min at 4°C and collected the supernatant and used it as enzyme. Prepared the 3ml reaction mixture by adding 200µl methionine, 100 µl EDTA, 100 µl NBT, 100 µl sodium carbonate solution, 1.5 ml phosphate buffer (0.1M), sample volume 100 µl, riboflavin100 µl and make up the volume to 3 ml by distilled water. One reaction mixture kept at dark and another kept under sunlight until the colour of the reaction mixture turned to blue colour i.e. 10 min. Prepared one set of reaction mixture without enzyme for both light and dark reaction and it used as control. It was known as 100% inhibition. Read the absorbance at 560 nm immediately, dark reaction sample used as a blank for their respective sample [19]. SOD activity was calculated by below formulae:

\[
\text{Superoxide Dismutase activity} = \frac{\text{Without Enzyme absorbance (Control)}}{\text{With enzyme Absorbance (Sample)}} \times 100 - 100
\]

\[
50\% \text{ inhibition of SOD activity (U/mg protein)} = \frac{\text{Concentration of sample (µg)}}{\text{SOD activity of sample}} \times 50
\]

Native PAGE was also carried out based on Sambrook and Russell (2001). For staining the gel prepared solution A which contains 20 ml of NBT (0.1%), 500 µl of EDTA (0.2 M), 50 µl of phosphate buffer (100 mM, pH 7.4) and 330 µl of TEMED; Solution B as 30 µl of riboflavin (0.005%). Staining mixture was prepared by adding both solution A and Solution B simultaneously in plastic gel tray. Incubate the gel inside the plastic gel tray containing combination of staining mixture for 15 min in dark without expose to direct light. After 15 min gel was exposed to the light for 3-5 min. Whitish coloured bands were observed with dark bluish background.

**Chlorophyll content in terrestrial microalgae**

The chlorophyll is the important components and it contains chloroplasts green pigment in photosynthetic plant tissues. The maximum chlorophyll a, b, c and total chlorophyll content was found in *Rhizoclonium hieroglyphicum* collected from Kaggodu (Kodagu district) as 14.82 ± 0.039, 12.42 ± 0.007, 7.11 ± 0.002 and 13.85 ± 0.087 mg/g tissue, respectively. The minimum chlorophyll a, b and total chlorophyll content was seen in *Rhizoclonium sp.* (Dandiganahalli) as 3.31 ± 0.002, 3.55 ± 0.151 and 3.78 ± 0.011 mg/g tissue, respectively. The minimum chlorophyll c
of 0.93 ± 0.014 mg/g tissue was observed in \textit{Rhizoclonium} sp. (Happanadka). In current study chlorophyll a, b, c and total chlorophyll content in \textit{Rhizoclonium hieroglyphicum} has 77.63, 71.41, 80.78 and 72.68% more than \textit{Rhizoclonium} sp. (Happanadka) (Table 3). 

The results of present study are in accordance with the similar study done by Benemann et al. (1978) \textsuperscript{[2]} and Ismaiel et al. (2016) \textsuperscript{[7]} who observed 10.6 mg/g of Chl a. The reaction centers of PSI and PSII in chloroplast thylakoids are the major generation site of reactive oxygen species (ROS). The photo production of ROS is largely affected by physiological and environmental factors \textsuperscript{[1]}. 

**Protein content in terrestrial microalgae**

In the current study total protein were estimated by Lowry method. Terrestrial microalgae \textit{Rhizoclonium} sp. (Happanadka), \textit{Rhizoclonium} sp. (Dandiganahalli), \textit{Spirogyra} sp., \textit{Klebsormidium} sp., \textit{Rhizoclonium hieroglyphicum} and \textit{Oedogonium} sp. had 9.93 ± 0.03, 22.24 ± 0.03, 6.64 ± 0.03, 5.12 ± 0.16, 19.51 ± 0.04 and 5.73 ± 0.02 mg/g of protein, respectively.

![Fig 2: SDS-PAGE for protein content of terrestrial microalgae](image)

Where M. Marker (9-235 kDa); 1,2,3,4,5 and 6 indicate the sample in that well are \textit{Rhizoclonium} sp. (Happanadka), \textit{Rhizoclonium} sp. (Dandiganahalli), \textit{Spirogyra} sp., \textit{Klebsormidium} sp., \textit{Rhizoclonium hieroglyphicum} (Kaggodu) and \textit{Oedogonium} sp., respectively. In SDS- PAGE, bands for all the samples were observed in 41 kDa, 30 kDa and 22 kDa region in the gel indicating the terrestrial microalgae proteins as 41kDa, 30kDa and 22kDa of molecular weight (Fig. 2). The results of present study are in accordance with the similar study done by Peter \textit{et al.}(2010) \textsuperscript{[14]} who correlated higher protein content with high lipid content in the microalgae.

**Carbohydrate degrading enzyme (Amylase enzyme)**

The amylases are common hydrolytic enzymes which hydrolyze or processing the starch \textsuperscript{[6, 21]} Endoamylases, Exoamylases, Debranching enzymes and Transferases are the four types of starch converting enzymes. Most starches are mixture of two polymers such as amylose and amylopectin \textsuperscript{[12, 24]}. Ratio of these two polymers in starch varies due to origin, plant species, variety within plants, plants organs, age of organ, and growth conditions \textsuperscript{[11, 23]}. In present study amylase enzyme was estimated in terrestrial microalgae of \textit{Rhizoclonium} sp. (Happanadka), \textit{Rhizoclonium} sp. (Dandiganahalli), \textit{Spirogyra} sp., \textit{Klebsormidium} sp., \textit{Rhizoclonium hieroglyphicum} and \textit{Oedogonium} sp. are 30.049 ± 0.015, 30.620 ± 0.022, 30.217 ± 0.042, 26.383 ± 0.004, 30.553 ± 0.015, 30.116 ± 0.065 and 27.939 ± 0.031 U/mg of maltose, respectively. The maximum amount of amylase enzyme was observed in \textit{Rhizoclonium} sp. (Dandiganahalli) 30.620 ± 0.022 U/mg of maltose and minimum in \textit{Klebsormidium} sp. 26.383 ± 0.004 U/mg of maltose (Table 4).

The carbohydrate digestive amylase enzyme activities in microalgae were confirmed by native- PAGE appearing whitish band under violet blue background (Fig. 3). In that native-PAGE 2nd well \textit{Rhizoclonium} sp. (Dandiganahalli) sample showed bright band compared to other indicating higher amylase activity that \textit{Rhizoclonium} sp. (Dandiganahalli) as more amylase enzyme activity. The result of present study is in accordance with the similar study done by Rodrigues (2017) \textsuperscript{[17]}.

**Table 3: Chlorophyll content of terrestrial microalgae studied**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chlorophyll a (mg/g tissue)</th>
<th>Chlorophyll b (mg/g tissue)</th>
<th>Chlorophyll c (mg/g tissue)</th>
<th>Total chlorophyll (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.36 ± 0.008</td>
<td>5.54 ± 0.008</td>
<td>0.93 ± 0.014</td>
<td>6.32 ± 0.005</td>
</tr>
<tr>
<td>B</td>
<td>3.31 ± 0.002</td>
<td>3.55 ± 0.151</td>
<td>1.37 ± 0.004</td>
<td>3.78 ± 0.011</td>
</tr>
<tr>
<td>C</td>
<td>4.14 ± 0.011</td>
<td>4.38 ± 0.122</td>
<td>1.94 ± 0.019</td>
<td>4.68 ± 0.014</td>
</tr>
<tr>
<td>D</td>
<td>7.81 ± 0.011</td>
<td>6.49 ± 0.011</td>
<td>4.03 ± 0.005</td>
<td>7.25 ± 0.045</td>
</tr>
<tr>
<td>E</td>
<td>14.82± 0.039</td>
<td>12.42± 0.007</td>
<td>7.11 ± 0.002</td>
<td>13.85± 0.087</td>
</tr>
<tr>
<td>F</td>
<td>3.97 ± 0.005</td>
<td>4.10 ± 0.001</td>
<td>2.15 ± 0.003</td>
<td>4.39 ± 0.010</td>
</tr>
</tbody>
</table>

All the values are MEAN ± SD of three replicates

\(A\) \textit{Rhizoclonium} sp. (Happanadka), \(B\) \textit{Rhizoclonium} sp. (Dandiganahalli), \(C\) \textit{Spirogyra} sp. (Hoovnalli), \(D\) \textit{Klebsormidium} sp. (Ikola), \(E\) \textit{Rhizoclonium hieroglyphicum} (Kaggodu) and \(F\) \textit{Oedogonium} sp. (Gadanahalli).

**Table 4: Amylase enzyme estimated from terrestrial microalgae**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Amylase (U/mg maltose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>30.049 ± 0.015</td>
</tr>
<tr>
<td>B</td>
<td>30.620 ± 0.022</td>
</tr>
<tr>
<td>C</td>
<td>30.217 ± 0.042</td>
</tr>
<tr>
<td>D</td>
<td>26.383 ± 0.004</td>
</tr>
<tr>
<td>E</td>
<td>30.553 ± 0.015</td>
</tr>
<tr>
<td>F</td>
<td>30.116 ± 0.065</td>
</tr>
<tr>
<td>G</td>
<td>27.939 ± 0.031</td>
</tr>
</tbody>
</table>

All the values are MEAN ± SD of three replicates

\(A\) \textit{Rhizoclonium} sp. (Happanadka), \(B\) \textit{Rhizoclonium} sp. (Dandiganahalli), \(C\) \textit{Spirogyra} sp. (Hoovnalli), \(D\) \textit{Klebsormidium} sp. (Ikola), \(E\) \textit{Rhizoclonium hieroglyphicum} (Kaggodu) and \(F\) \textit{Oedogonium} sp. (Gadanahalli)

**Antioxidant activities in terrestrial microalgae**

**DPPH (2, 2-diphenyl-1-picrylhydrazyl) activity**

DPPH radical is the stable organic nitrogen free radicals, which used to determine the free radical scavenging ability of the various samples \textsuperscript{[15]}. DPPH is a purple color dye having absorption maxima of 517 nm and upon reaction with a hydrogen donor the purple color fades or disappears due to conversion of it to 2, 2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance\textsuperscript{[26]} In present study DPPH (2, 2-diphenyl-1-picrylhydrazyl) activity were estimated in terrestrial microalgae of \textit{Rhizoclonium} sp. (Happanadka), \textit{Rhizoclonium} sp. (Dandiganahalli), \textit{Spirogyra} sp., \textit{Klebsormidium} sp., \textit{Rhizoclonium hieroglyphicum} and \textit{Oedogonium} sp. had 28.97 ± 2.069, 40.00 ± 3.006, 41.38 ± 2.068, 38.62 ± 1.379, 44.14 ± 1.194 and 42.07 ± 0.689% of radical scavenging activity (% RSA) respectively (Table 5).

Free radicals scavenging potential was assayed by DPPH...
method and were expressed in % radical scavenging activity (% RSA). DPPH method gives antioxidant activity which helps to understand the electron abstraction potential of microalgae. The highest % of RSA was found in *Rhizoclonium hieroglyphicum*. The antioxidant activities (the radical scavenging activity, reducing power and chelating activity) were enhanced under stress conditions and at the pH level that supports optimal growth. The % RSA observed in present study are statistically significant. The results of present study are in accordance with the similar study done by Maadane et al. (2015) and Trabelsi et al. (2016) on microalgae of different species.

** Peroxidase activity**

In microalgae an increase in the activity of these enzymes not only promotes ROS removal, but also increases ATP synthesis via the Mehler peroxidation reaction which is the source of additional ATP for Na⁺ expulsion at high salinity. POD activities were estimated in terrestrial microalgae of present studied samples are 0.01 ± 0.003, 0.21 ± 0.004, 0.19 ± 0.008, 0.19 ± 0.002, 0.21 ± 0.004 and 0.01 ± 0.001 µmole/min/g weight of sample, respectively (Table 5). The antioxidants produced by algae alleviate the harmful effects of ROS. In present study monitored the enzyme activities of algae in various present studied species. The highest peroxidase activity was observed in *Rhizoclonium sp.* (Dandiganahalli) and *Rhizoclonium hieroglyphicum* are 0.21 ± 0.004 µmole/min/g and lowest in *Oedogonium sp.* are 0.01 ± 0.001 µmole/min/g weight of sample. POD activities in microalgae were confirmed by conducting native-PEGE for POD and brown coloured bands were observed. *Rhizoclonium sp.* (Dandiganahalli) and *Rhizoclonium hieroglyphicum* (Kaggodu) samples shows bright bands in native-PAGE gel because of high POD activity *Spirogyra sp.* and *Klebsormidium sp.* samples showed light band because of low POD activity (Fig. 3). The result of present study is in accordance with the similar study done by Chakraborty et al. (2010).

** Superoxide dismutase (SOD) activity**

One unit of SOD activity were defined as the corrected amount of enzyme (by the negative control) required to result in a 50% inhibition of the Nitroblue tetrazolium (NBT) reduction measured at 560 nm in comparison with the positive control under the assay conditions described. The activities were expressed in U/mg protein. In present studied sample as SOD activities of 124.40 ± 0.211, 97.98 ± 1.025, 186.29 ± 2.205, 168.66 ± 0.131, 92.23 ± 1.029 and 282.95 ± 0.036 U/mg proteins, respectively was noted (Table 5). In present study the highest SOD activity was observed in *Rhizoclonium hieroglyphicum* because it needs only 92.23 ± 1.029 U/mg protein for 50% inhibition of enzyme (IC₅₀) and lowest was observed in *Oedogonium sp.*, it was needed 282.95 ± 0.036 U/mg protein for IC₅₀ SOD, CAT and POD are most important antioxidant enzymes in the algal cell that protect against the peroxidation system and maintain the redox state of the cell. The scavenging of ROS not only protects chloroplasts from the direct effects of ROS, but also relaxes the photon (electron) excess stress. The SOD activity in microalgae was confirmed by native-PEGE for SOD and observed whitish bands under dark bluish background (Fig. 3).

**Table 5: Antioxidant activities in terrestrial microalga**

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>DPPH (% RSA)</th>
<th>POX (µmole/min/g)</th>
<th>SOD (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>28.97 ± 2.069</td>
<td>0.01 ± 0.003</td>
<td>124.40 ± 0.211</td>
</tr>
<tr>
<td>B</td>
<td>40.00 ± 3.006</td>
<td>0.21 ± 0.004</td>
<td>97.98 ± 1.025</td>
</tr>
<tr>
<td>C</td>
<td>41.38 ± 2.068</td>
<td>0.19 ± 0.008</td>
<td>186.2 ± 2.205</td>
</tr>
<tr>
<td>D</td>
<td>38.62 ± 1.379</td>
<td>0.19 ± 0.002</td>
<td>168.66 ± 0.131</td>
</tr>
<tr>
<td>E</td>
<td>35.86 ± 3.160</td>
<td>0.07 ± 0.002</td>
<td>178.91 ± 0.096</td>
</tr>
<tr>
<td>F</td>
<td>44.14 ± 1.194</td>
<td>0.21 ± 0.004</td>
<td>92.23 ± 1.029</td>
</tr>
<tr>
<td>G</td>
<td>42.07 ± 0.689</td>
<td>0.01 ± 0.001</td>
<td>282.95 ± 0.036</td>
</tr>
</tbody>
</table>

All the values are MEAN ± SD of three replicates.

**Conclusion**

Terrestrial microalgae collected from Western Ghats were adjusted to grow in RO residual dispense water with pH 8.4 and urban waste water. *Rhizoclonium sp.* and *Rhizoclonium hieroglyphicum* collected from Dandiganahalli and Kaggodu, respectively grown very quickly and which reduces the pH of waste water from alkaline to neutral or acidic and also reduces the TDS content of water. On the basis of above investigation it can be concluded that the terrestrial microalgae can be used for bioremediation purpose of alkaline water. Amylase enzyme activity was high in *Rhizoclonium sp.* (Dandiganahalli) which can be used in industries for carbohydrate degrading purpose in future. *Rhizoclonium sp.* (Dandiganahalli) and *Rhizoclonium hieroglyphicum* (Kaggodu) showed good response to antioxidant properties to survive in stress. Based on study conducted, SOD, POD and DPPH were the scavenger of ROS protecting the chloroplast from direct effect of ROS and also protecting the cell from aging effects. This may be the reasons for microalgae were able to survival in various environmental factors.

**Acknowledgement**

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