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RESEARCH ARTICLES

Microalgal biomass generation by phycoremediation of sewage water: an integrated approach for production of antioxidant and value added products

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Abstract

Sewage water from an urban township in India has been used as a growth media for cultivation of a green alga *Chlorella ellipsoidea* in relation to phycoremediation and integrated approach for production of antioxidant and value added products. The concentration level of nitrate, phosphate, ammonia and total dissolved solid got significantly reduced in the sewage water media at post-stationary phase. An increased level of dissolved oxygen was observed on 30th day of incubation. The alga was efficient in the uptake of nutrients from sewage water. Most notably, complete removal of total dissolved solid was observed. Phaeopigment and physiological stress indices were virtually unaltered in most cases indicating facile adaptability. The stationary phase of the algal species was stable for 40 days as against only 22 days in BG11(N+) medium. Dry cell weight of the sewage water grown alga has been found to be about five times higher than that grown in BG11(N+) medium. The biomass accrued from sewage water as growth medium showed around 25% carbohydrate, 35% lipid and 56% protein and relatively higher amount of enzymatic and non-enzymatic antioxidants.

Keywords *Chlorella ellipsoidea* · Sewage water · Nutrient uptake · Phycoremediation · Phaeopigment

Abbreviations

DO	Dissolved oxygen
BOD	Biological oxygen demand
COD	Chemical oxygen demand
TDS	Total dissolved solid
SS	Suspended solid
DCW	Dry cell weight

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Introduction

Algae are well recognised for their potential role in biotransformation of pollutants, nutrients and xenobiotic from waste water and carbon dioxide from waste air (Babaei et al. 2018). The release of wastewater to the receiving natural water bodies poses serious environmental challenges (De-Bashan and Bashan 2010). Microalgae by way of nutrient removal can help counter eutrophication in the aquatic ecosystem and are unique in sequestering carbon dioxide (Olguín 2003). The domestic sewage water being rich in organic carbon, nitrogen, phosphorus and other compounds supports the growth of microalgae (Liang et al. 2013).

Like fungi and bacteria, many algae are capable of sorbing heavy metals from polluted waters (Mehta and Gaur 2005; Yadav et al. 2017). Microalgae cultivation are now-a-days integrated with various wastewater as growth media to reduce the production cost of biomass. Apart from being cost-effective, recovering nutrients from waste water through microalgae assimilation is also considered a viable and sustainable option. Growing algae using sewage water (Renuka et al. 2013; Singh et al. 2017; Bansal et al. 2018) have been documented. The technique is considered efficient in producing high value added products such as proteins, carbohydrates, lipids, enzymatic, non-enzymatic antioxidants and biofuels as an alternative source of energy (Kumar et al. 2014; Marchão et al. 2017). Blend of organic and inorganic materials, sewage water generally contains carbohydrates, fats, proteins, amino acids, and volatile acids besides inorganic ions such as sodium, calcium, potassium, magnesium, chlorine, sulphur, phosphate, bicarbonate, ammonium salts and heavy metals (Tebbutt 1983). Silchar, a class II urban township in Assam state in India alone produces 8000 kilo litres of sewage water daily which is generally released into the river Barak (CPCB 1999; Datta Ray et al. 2000). Municipal wastewater is replete with pathogenic and non-pathogenic bacteria besides various toxin compounds (Shannon et al. 2007). Wastewater treatment by physico-chemical means are quite expensive, cumbersome and often not feasible. Thus low-cost effective biological waste treatment methods have drawn immense attention of researchers across the world (Yadav et al. 2017; Babaei et al. 2018). All algal species, however, cannot thrive in wastewater environment and selection of algal species is very crucial in the context of wastewater treatment (Pittman et al. 2011). The nutrient uptake potential of genus *Chlorella* from wastewater has been explored rather extensively (Megharaj et al. 1992; Marchão et al. 2017; Salgueiro et al. 2018).

Accordingly we report herein an assessment of growth of an algal species *Chlorella ellipsoidea* using raw sewage water of Silchar town located in the state of Assam, India in relation to phycoremediation, biomass production via an integrated approach of nutrient uptake process.

Materials and methods

Collection and analyses of domestic sewage water sample

Domestic sewage water samples were collected in clean polythene bottles from the apartment's outlets of Vivekananda road, Silchar town, Assam (Fig. 1a).The colour of the domestic sewage water was 'greyish' with BOD and COD values being 600 ± 0.18 mg/l and 1520 ± 0.18 mg/l. (Table 1), respectively. The samples were filtered to remove any suspended particles and stored at 4 °C until further use. Standard protocols were followed for analysis of domestic sewage water (APHA 2005).

Fig. 1 **a** Domestic sewage water collection site; **b** *Chlorella ellipsoidea*; **c** domestic sewage water without any pre-treatment; **d** phycoremediation treatment series

Table 1 Physico-chemical data of domestic sewage water

Parameters	Value
Colour	Greyish
Odour	offensive
Temperature	32 ± 0.56 °C
pH	8.3 ± 0.13
BOD	600 ± 0.18 mg/l
COD	1520 ± 0.18 mg/l
DO	2.2 ± 0.23 mg/l
Alkalinity	10 ± 1.4 mg/l
Free CO_2	36.98 ± 0.13 mg/l
TDS	500 ± 0.18 mg/l
Suspended solids	50 ± 0.54 mg/l
Chloride	60 ± 0.67 mg/l
Calcium	60 ± 0.13 mg/l
Sulphate	50 ± 1.6 mg/l
Nitrate	12 ± 1.5 mg/l
Magnesium	30 ± 0.02 mg/l
Ammonia	30 ± 0.12 mg/l
Phosphate	70 ± 0.45 mg/l

Microalgae and growth condition

Chlorella ellipsoidea (AUS/EC/JR/PSGA294) was collected from submerged polythene surface in domestic sewage water of Silchar town, Assam state in India and pure culture developed. The algal species (Fig. 1b) was maintained in nitrogen enriched BG11(N+) media under continuous illumination (2000 lx) at 24 ± 1 °C (Rippka et al. 1979). The isolated microalga was identified using standard keys (Prescott 1952). The *C. ellipsoidea* was grown in varying concentration of domestic sewage water with five treatments (Fig. 1c) including BG11(N+) media (Table 2). The microalga grown in BG11(N+) medium served as a control. The flasks for phycoremediation series in different amount of domestic sewage water is shown in Fig. 1d. Flasks were shaken twice a day to avoid adherence of cells at bottom surface of conical flask.

The culture was thoroughly vortexed and cell count was performed using a Neubauer haemocytometer. The chlorophyll *a* (Chl *a*) was determined spectrophotometrically (Strickland and Parsons 1968).

Table 2 Different treatment series used for phycoremediation of domestic sewage water

Treatment series	Code
BG11(N+)	a
Domestic sewage water	b
BG11(N+) (10%) + domestic sewage water (90%)	c
BG11(N+) (50%) + domestic sewage water (50%)	d
BG11(N+) (90%) + domestic sewage water (10%)	e

Nutrient removal analysis

The nutrient removal capacity of *C. ellipsoidea* was assessed at the first and last day of the experiment by measuring physico-chemical properties of domestic sewage water. The removal rate of NO_3^- and PO_4^{3-} were calculated as per equation: removal rate (mg L^{-1} day^{-1}) = $C_i - C_f/t$; where C_i and C_f are initial and final concentration of nutrients or DO and t is the time duration.

DCW of *C. ellipsoidea*

On 30th day of treatment, 10 ml of homogenised culture were filtered through pre-weighed Whatman filter (grade 42) papers and dried at 60 °C until constant weight of DCW was obtained.

Biochemical analysis of *C. ellipsoidea*

The total carbohydrate was determined according to anthrone method (Spiro 1966) and total protein was determined following modified folin's method (Herbert et al.1971). Lipid estimation has been carried out as per Bligh and Dyer (1959).

Estimation of enzymatic and non-enzymatic antioxidants

Catalase activity was estimated according to Aebi (1984). Peroxidase activity was assayed by the method of Kar and Mishra (1976). Superoxide dismutase (SOD) activity was determined as per method of Van Rossum et al. (1997). Glutathione peroxidase activity was estimated according to standard procedure (Rotruck et al. 1973). Ascorbic acid contents were determined as per Roe and Kuether (1943). Glutathione reductase was assayed by the method of Schaedle and Bassham (1977).

Phaeopigment and physiological stress indices

Physiological stress indices (chlorophyll to phaeophytin ratio) of *C. ellipsoidea* grown in domestic sewage water and domestic sewage water + BG11(N+) treatments were determined according to the standard method (Megateli et al. 2009).

Conversion of phaeophytin from chlorophyll was effected by addition of 10μL HCl (35% GR, Merck) to 3 ml of extract. The ratios, D_{430}/D_{410} (Phaeopigment Index), D_{430}/D_{665} (Margalef Index I), D_{480}/D_{665} (Margalef Index II) were evaluated (Martinez-Abaigar and Nùñez-Oliveira 1998).

Statistical analyses

Correlation study was performed using the Software Statistical Package for Social Sciences (SPSS Version 21.0). Data are average of three experiments ± S.D, n = 3.

Results and discussion

Physico-chemical properties of domestic sewage water

The colour of domestic sewage water was greyish (Table 1). The temperature was 32 ± 0.56 °C. BOD, COD and DO of water was 600 ± 0.18, 1520 ± 0.18 and 2.2 ± 23 mg/L, respectively. The total alkalinity was 10 ± 1.4 mg/L, free CO_2 was 36.98 ± 0.13 mg/L and TDS was found to be 500 ± 0.18 mg/L. The SS was 50 ± 0.54 mg/L. The chloride and calcium concentrations were found to be 60 ± 0.67 mg/L, 60 ± 0.13 mg/L, respectively. The sulphate, nitrate, magnesium and ammonia of sewage water was present at a concentration of 50 ± 1.6 mg/L, 12 ± 1.5 mg/L, 30 ± 0.23 mg/L and 30 ± 0.45 mg/L, respectively. The phosphate concentration was found to be 70 ± 0.45 mg/L. These results (Table 1) are in compliance with a recent study from this town (Sarmah and Rout 2017).

Effect of sewage water on cell number and Chl *a*

Growth study of *C. ellipsoidea* in different treatments (Fig. 2a) show the lag period and log period to be longest in raw domestic sewage water treatment. The treatment c showed a shorter lag period than that of raw sewage treatment. The treatment d, control BG11(N+) and the treatment e showed same log period. Stationary period was found to be the longest for the treatment b. Similar growth profiles were observed in the treatments d and e. Maximum cell number (1008×10^6cell/ml) was observed in the treatment b on 16th day of the treatment (Fig. 2). The cell number in domestic sewage water grown *C. ellipsoidea* were considerably higher than that of BG11(N+) grown culture. This suggests that *C. ellipsoidea* can grow well in sewage water without additional nitrogen source. This is in conformity with another species of *C. vulgaris* which showed higher growth in confectionery effluent water (Kumar et al. 2014).The *C. ellipsoidea* was found growing till 40 days, while the standard BG11(N+) medium registered growth till 22 days from inoculation. A wild-type *Chlorella* sp. isolated from freshwater was found to grow, adapt easily in diluted dairy manure samples in comparison to TAP media (Wang et al. 2010). In a related study, *Chlorella* sp. grown in municipal sewage water, 33% decline in growth rate was observed in comparison to standard media (Mutanda et al. 2011).

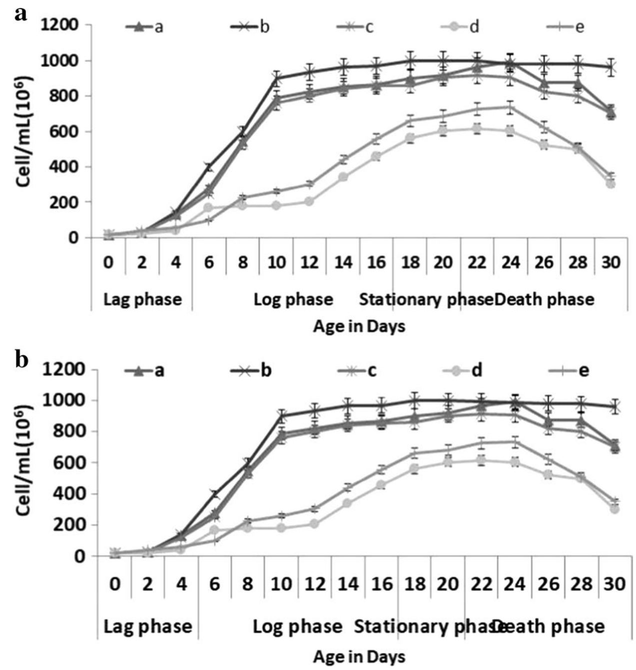


Fig. 2 Effect of domestic sewage water on **a** cell number and **b** Chl *a* of *C. ellipsoidea*

In this study, the peak point of growth of *C. ellipsoidea* was observed on day 16 of cultivation. The Chl *a* content was highest in the treatment b (raw sewage water), lowest in e (Fig. 2b). Due to high nutrient content, sewage water used in the present study may serve as a suitable media for cultivation of *C. ellipsoidea*. The pH was monitored daily for the different treatment series for a period of 30 days (Fig. 3). The alga reduced the dissolved CO_2 concentrations of sewage water through photosynthesis which in turn raised the pH level. The growth of alga was affected by pH. High pH can lower the growth of the microalgae besides inhibiting the photosynthetic rate of the alga (Leavitt et al. 1999). The algal growth was measured in terms of Chl *a* content. A positive correlation was noted for the residual phosphate ($r = 0.974^{**}$), nitrate (0.765^{**}), ammonia (0.532^{**}) and TDS

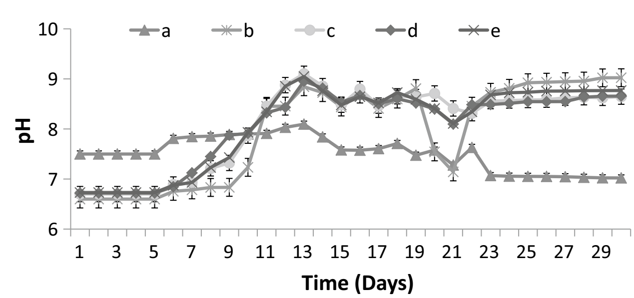


Fig. 3 Effect of domestic sewage water on pH in the different treatments for a period of 30 days

Table 3 Bivariate correlation analysis of the nutrients removal and algal growth conditions

	Chl *a*	pH	Nitrate removal	Ammonia removal	Phosphate removal	DO increased	TDS removal
Correlations							
Chl *a*	1						
pH	0.479**	1					
Nitrate removal	0.765**	0.749**	1				
Ammonia removal	0.532**	0.987**	0.373*	1			
Phosphate removal	0.974**	0.779**	0.147	0.467*	1		
DO increased	0.438*	0.934**	0.312**	0.134	0.693**	1	
TDS removal	0.768**	0.340*	0.501*	0.693**	0.072	0.285*	1

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

(0.768**) and increased rate of DO (0.438*) in the medium with the Chl *a* concentration of the culture (algal biomass) (Table 3).

DCW of *C. ellipsoidea*

The DCW was quite higher (39.67 mg L^{-1}) in sewage water compared to BG11(N+) medium (18.67 mg L^{-1}) (Table 4), reflecting a significant increase in DCW (50%) in domestic sewage water in 4th day of the treatment. For 30th day of the treatments also, the dry cell weights in sewage water (998 mg L^{-1}) were found to be higher compared to BG11(N+) medium (718 mg L^{-1}). In a recent study, *Parachlorella kessleri* grown in municipal waste water has been shown to produce 50% more biomass as compared to TAP and ASW media (Singh et al. 2017).

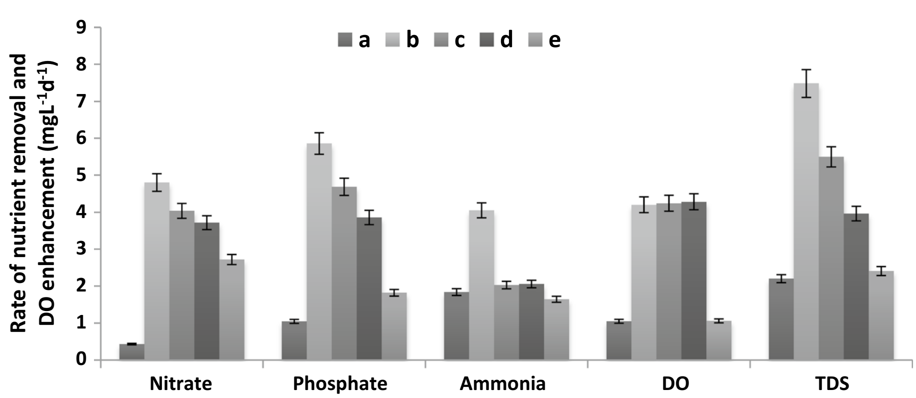
Table 4 Dry cell weight (mg L^{-1}) of *C. ellipsoidea* in the different treatments

Treatment	4th day	30th day
a	18.67 ± 0.24	178 ± 1.2
b	39.67 ± 0.56	998 ± 0.89
c	28.67 ± 0.78	913 ± 0.93
d	25.67 ± 0.12	893 ± 0.76
e	24.67 ± 0.34	819 ± 1.45

Removal rate of nitrate, phosphate and ammonia

Nitrate, phosphate and ammonia removal rates were found to be maximum (4.79, 5.85, 4.05 mg L^{-1} day^{-1}, respectively) in the treatment b (raw sewage water) as compared to BG11 (N+) media (Fig. 4). It is generally believed that there is direct correlation between chlorophyll production and nutrients removal efficiencies of alga in sewage water. In the present case, both parameters recorded highest in log period of *C. ellipsoidea*. The nutrient sequestration pattern suggests that removal efficiencies were associated with algal growth (Kim et al. 2013; Delgadillo-Mirquez et al. 2016; Babaei et al. 2018).This can be ascribed to nitrogen dependence of *C. ellipsoidea* from sewage water and large surface area and high binding affinity of algae. Cell wall of algae is considered as main binding site for nutrients and metals

Fig. 4 Rate of nutrient removal and DO enhancement by *C. ellipsoidea* in different treatments on the 30th day of incubation



(Romero-Gonzalez et al. 2001). Phosphate removal rate was very high presumably owing to the utilization of phosphorus for their growth.

DO of sewage water

Dissolved oxygen, an important water quality parameter was found to have significantly high rate of enhancement (4.2 mg L^{-1} day^{-1}) in the treatment b (raw sewage water) (Fig. 4). About 46% hike has earlier been observed in DO concentration in sewage water when incubated with *Calothrix* sp. (Renuka et al. 2013). In another recent study, 96% increase in DO rate was observed for *Scenedesmus* sp. in tannery waste water (Ajayan et al. 2015). It is evident that increased rate of DO associated with reduced dissolved CO_2 concentrations through photosynthesis raises the pH level (Borowitzka 1998).

Removal rate of TDS

The maximum TDS removal rate (7.48 mg L^{-1} day^{-1}) was observed in the treatment b, raw domestic sewage water, as compared to BG11(N+) media (Fig. 4). Notably, the species *C. ellipsoidea* in the present study, showed the potential to reduce the TDS below permissible limit. The TDS removal was found to be 41% for *C. vulgaris* in tannery wastewater (Das et al. 2017).

Phaeopigment and physiological stress

Stress condition in different plants such as algae (Marker et al. 1980), bryophytes (Spitale 2009), and vascular plants (Megateli et al. 2009) were calculated by measuring photosynthetic pigment composition. Photosynthetic pigment composition was calculated at the end day of the sewage water phycoremediation. Phaeopigment, Margalef I and Margalef II indices were calculated (Fig. 5). For the different treatments, phaeopigment and physiological stress indices revealed a trend of a > c > d > b > e. The Magalef index I and

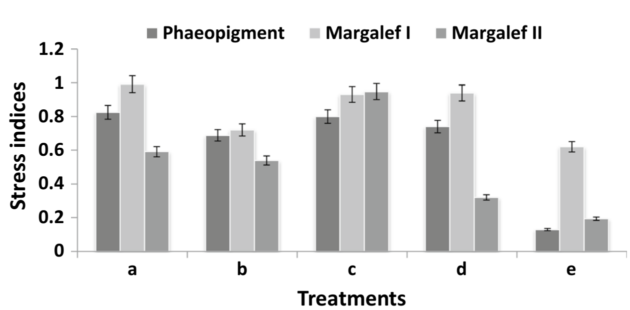


Fig. 5 Influence of different treatments on the phaeopigment and physiological stress indices of *C. ellipsoidea* on the 30th day of inoculation

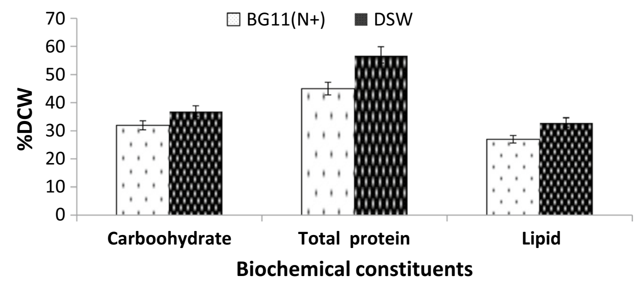


Fig. 6 Biochemical composition of *C. ellipsoidea* grown in BG11(N+) medium and domestic sewage water

II also showed a similar trend indicating virtually no stress level for the alga. Similar low stress levels were found in phycoremediation of sewage water by *Chlorella* sp. (Renuka et al. 2013).

Biochemical contents

The carbohydrate content (35 ± 0.72%) of *C. ellipsoidea* grown in sewage water was higher compared to that of the BG11(N +) grown (30 ± 0.81%). Total protein content (56 ± 0.77%) of *C. ellipsoidea* grown in domestic sewage water was also higher than that of the BG11(N+) grown (43 ± 0.54%). Lipid content of *C. ellipsoidea* grown in sewage water (34 ± 0.67%) was relatively higher than that of the BG11 (N+) grown (24 ± 0.52%, Fig. 6). It is noteworthy that biochemical constituents of *C. ellipsoidea* were obtained 2–3 times higher as compared to those of BG11(N+) media maintained culture. The alga, *Acutodesmus dimorphus* was found to contain around 25% lipid and 30% carbohydrate (Chokshi et al. 2016). For *C. sorokiniana*, cultivated in municipal sewage water, an enhanced amount of biomass (77.14 mg L^{-1} day^{-1}), lipid (24.91 mg L^{-1} day^{-1}), carbohydrate (20.10 mg L^{-1} day^{-1}) and protein (22.36 mg L^{-1} day^{-1}) were observed (Ramsundar et al. 2017).

Enzymatic and non-enzymatic antioxidants

The sewage water grown *C. ellipsoidea* showed 56U/mg of protein of catalase, 54U/mg of protein of peroxidase, 63U/mg of protein of superoxide dismutase, 53U/mg of protein of glutathione peroxidase and the laboratory maintained *C. ellipsoidea* showed 53 U/mg of protein of catalase, 51 U/mg of protein of peroxidase, 60 U/mg of protein of superoxide dismutase and 47 U/mg of protein of glutathione peroxidase. Non-enzymatic antioxidants, 2.5 µg/mg of ascorbic acid, 69 U/mg of protein of glutathione reductase were recorded in *C. ellipsoidea* grown in sewage water. Ascorbic acid and glutathione reductase content in control were 1.18 µg/mg and 61 U/mg of protein, respectively. In a recent study, *C. vulgaris* cultivated in effluent of a confectionery waste water revealed similar results (Kumar et al. 2014). The non-enzymatic antioxidants were shown to be highly crucial in scavenging reactive oxygen species (ROS).

Conclusion

Cultivation of *C. ellipsoidea* algae in untreated municipal sewage water without any additional nutrients afforded remarkable growth and biomass production. The lipid rich green alga was able to effectively sequester nitrate and phosphate, increase the DO level, and lower the TDS well below the permissible limit. The study also demonstrate that the microalga is capable of efficiently remediate domestic sewage water, mitigate carbon dioxide as it grow proficiently in polluted water. High lipid production in sewage water relative to the control medium may be exploited for its feasibility in biofuel generation. The antioxidant produced by the alga during the remediation of domestic sewage water is anticipated to be of significance in pharmaceutical, food and cosmetic applications. Employing domestic sewage water to harvest algae for production of value added chemicals could thus serve as an integrated approach for manifold applications.

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Data availability All data generated or analyzed during this study are included in this published article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights There is no research involving human participants and or animals.

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