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EFFECT OF HIGH HYDROSTATIC PRESSURE ON THE CELL WALL OF *POLYSIPHONIA NIGRESCENS* AND *P. URCEOLATA* FROM BALTIC SEA.*

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Abstract

The post-treatment effects of hydrostatic pressure on the cell-wall of *Polysiphonia nigrescens* (Huds.) Grev. and *P. urceolata* (Dillw.) Grev., collected from different depths of Kieler Förde, were studied. The upper and lower limits of tolerance were found to differ in both of them. *P. urceolata*, the shallow-water alga was more pressure sensitive than *P. nigrescens*, the deep-water type. Short exposures to 500 atm produced black spots and circles on the cell-wall, whereas greater exposures caused a swelling of the cell-wall. The cell-morphological abnormalities became severe with the increase of hydrostatic pressure as well as the duration of pressure action. Cellular abnormalities produced after short pressure exposures gradually disappeared, but the exposures to 800 atm rendered irreversible thickening of the cell-wall resulting in ultimate death of the algae. The exposures to 1000 atm were lethal for the survival of the treated organisms.

Introduction

Marine algae, usually being the photo-autotrophic organisms, are restricted in their occurrence to euphotic zone, where hydrostatic pressures of more than a few atm are never experienced. This probably explains the lack of interest in the studies of pressure effects on the biology of marine algae. With the discovery of living Diatoms (Wood, 1956), Coccolithophores (Bernard, 1963), some heterotrophic bathypelagic phytoplankters (Steemann-Nielsen, 1960; Kimball *et al.*, 1963), Chrysomonads, Dinoflagellates and certain genera of Volvocales and Cyanophyceae (Bernard, 1967; Vidaver, 1972) in the aphotic zone, as far down as 4000 m below the surface of the ocean, the study of hydrostatic pressure becomes extremely important on the celluiar morphology, development and physiology of the marine algae.

There are hardly any studies concerning the effect of hydrostatic pressures on the biology of phytoplankton, although they are highly significant primary producers of the hydrosphere. Investigations about the pressure effects on morphology, reproduction, growth and metabolism of benthic algae *i.e.* at the organismal level are however a few (Shameel, 1973c), more researches are therefore required specially at the molecular level, if adaptative functions which permit life to occur in the deep-sea are to be explained. The present paper deals with the observations made on the hydrostatic pressure effects on the cell-wall of two benthic species of a marine genus, *Polysiphonia* belonging to Ceramiales (Rhodophyceae).

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*Dedicated to the memory of my teacher, Prof. Fritz Gessner on his first death-anniversary on 20-12-1973.

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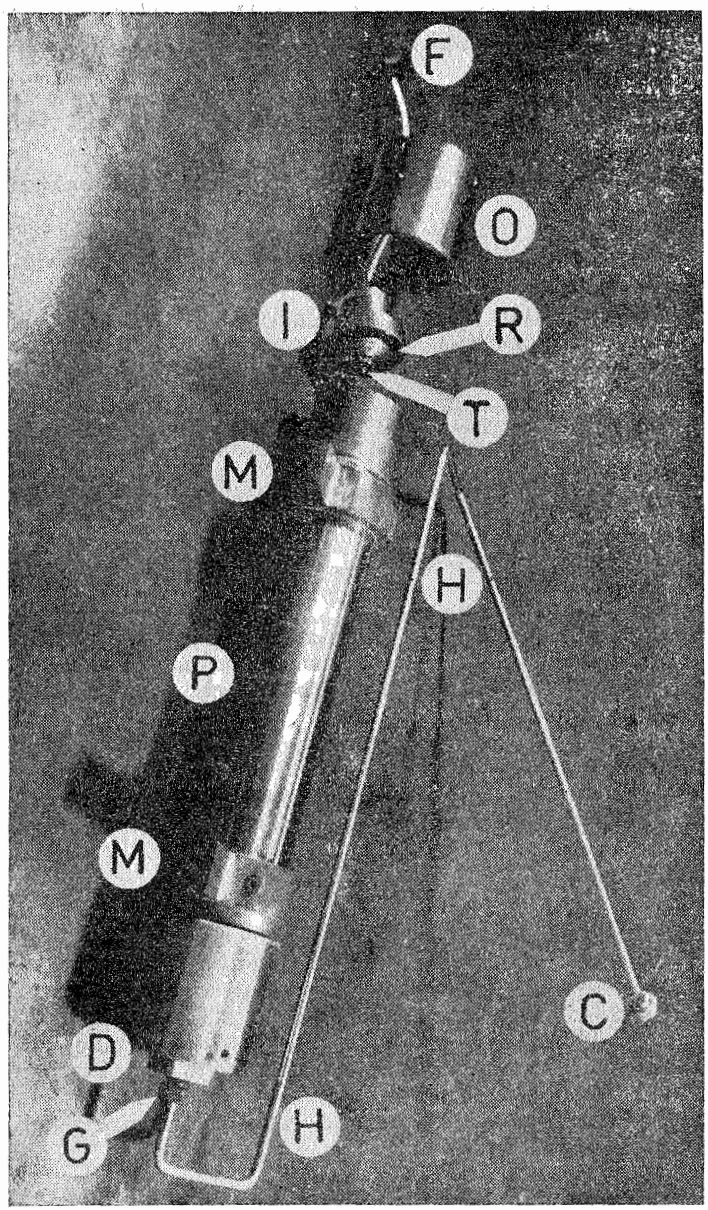

Fig. 1. Pressure chamber opened from one side, C — connected with pressure pumping system, D — double cap, F — for temperature measurement, G — gland nut & plug, H — hollow wire, I — inner cap, M — mechanical support for cylinder, O — outer cap, P — pressure cylinder, R — rubber-O-ring, T — thermistors.

Materials and Methods

Of a number of species of *Polysiphonia* reported from "Kieler Förde" (western Baltic Sea), *P. urceolata* (Dillw.) Grev., a shallow-water form and *P. nigrescens* (Huds.) Grev. a deep-water type were selected for the study. The former was collected from big pebbles near Light House (Kieler Innenförde) and the latter was dredged from 8—10 m depth at "Tonne C"(Kieler Aussenförde) from the board of R.B. "Sagitta". The algae were determined after Lakowitz (1929) and Kylin (1944). They were brought in thermos flasks together with the sea-water of their occurrence within a few hours to the constant-temperature rooms. The experimental sea-water was always adjusted to 15‰ which corresponds with the average salinity in Kiel Bight (Gessner, 1957). The procedure and the technique of algal culture were the same as described earlier (Shameel, 1973b).

After keeping the algae for 2 weeks' gradual adaptation to 15°C, since it can be easily tolerated by *Polysiphonia* without any after-effects (Schwenke, 1959), thalli which were uninjured and free from epiphytes were selected. They were then subjected to hydrostatic pressure, the pressure levels used were 400, 500, 800 and 1000 atm. A change in the thallus weight was observed in *P. urceolata* after subjecting it to pressures higher than 500 atm (Shameel & Ohno, 1972), therefore a minimum of 400 atm was selected for the present study. Three experimental series of 5, 10 and 20 hours' duration were used. For this purpose the algal material (A) was kept together with their culture medium in well-stoppered sample tubes (S), which were then placed in a pressure chamber (Figs. 1& 2). This chamber was designed by the author on the principles used by ZoBell (1959) for his studies on deep-sea bacteria. It was constructed in the workshop of "Institut für Meereskunde, Kiel".

The pressure chamber has a stainless steel cylinder (P), 400 mm high with 11 mm wall thickness, its inner volume being approx. 550 ml (Fig. 2). It can be opened from both the sides by means of a double cap (D). The outer cap (O) is a hollow cylinder of 80 mm in height and 59.5 mm inner diameter, and has 10.25 mm wall thickness and 50 mm long screws. The roof of this cap is partly closed leaving a central hole of 40 mm in diameter, from which the inner cap (I) projects out. This cap has a convex lower surface, so that no air-bubble may accumulate in the chamber. On the side of the lower surface there is a rubber-O-ring (R), which expands during pressure application and tightens the chamber. Through the lower inner-cap enters the hollow wire (H) of 304-stainless steel measuring 1/4 inches in outer and 3/32 inches in inner diameter, through which pressure is introduced or released. From the upper inner-cap hangs a projection (T) containing thermistors, which are connected (F) to a temperature-recorder for constant recording during pressure application. The double-cap system has an advantage over chambers designed similarly (Zimmerman, 1970; Kinne, 1972; Theede, 1973) in that the inner area of the chamber is increased as well as the parts are easily replaceable. The chamber is placed in a water bath (B), in which temperature-controlled water (W) constantly circulates.

The pressure cylinder (P) is connected (C) via a double-needle valve to a hydraulic hand pump (Fig. 1). The details of the pressure apparatus and the techniques employed have already been described (Shameel, 1973a; Shameel & Ohno, 1972). Ordinary tap-water was used as hydraulic fluid. During pressure operation the control algae were kept in an aluminium chamber under similar experimental conditions but at normal atmospheric pressure. After pressure release the algae were immediately checked

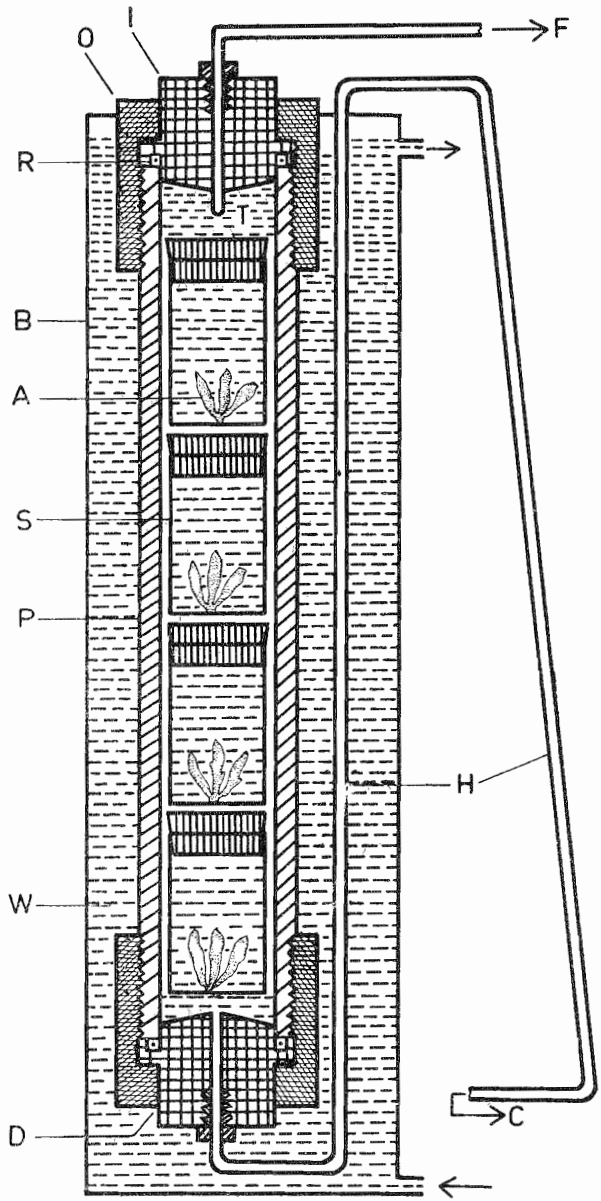

Fig. 2. Diagrammatic longitudinal section through the pressure chamber, A — algal material, B — water bath, C — connected with pressure pumping system, D — double cap, F — for temperature measurement, H — hollow wire, I — inner cap, O — outer cap, P — pressure cylinder, R — rubber-O-ring, S — sample tube with rubber stopper, T — thermistors, W — cooling water in circulation.

under microscope mounted in culture medium. The cellular morphology of the pressure treated algae was regularly and periodically examined upto 3 weeks and compared with the controls.

Results

1. OBSERVATIONS IMMEDIATELY AFTER PRESSURE RELEASE

The application of a hydrostatic pressure of 400 atm caused no effect in both the species at all the three durations of pressure action (Table 1). But after a pressure treatment of 500 atm for 5 hrs, *P. urceolata* exhibited some peculiar type of black spots and circles on cell-wall of the entire thallus, whereas *P. nigrescens* remained without any change after similar treatment. When the pressure duration was increased to 10 hrs, *P. urceolata* showed a peculiar swelling of cell-walls in the entire thallus, while *P. nigrescens* showed black spots and circles on the cell-wall. There was no change in the control algae

TABLE 1. Effect of hydrostatic pressure applied for different durations (in hours) at 15°C on the cell wall of *Polysipohnia spp.* as observed immediately after pressure release.

Pressure applied (atm)	5 hours	10 hours	20 hours
P. urceolata			
400	no effect	no effect	no effect
500	black spots	thick walls	thick walls
800	thickening irreversible	thickening irreversible	thickening irreversible
1000	dead cells	dead cells	dead cells
P. nigrescens			
400	no effect	no effect	no effect
500	no effect	black spots	thick walls
800	thick walls	thickening irreversible	thickening irreversible
1000	thickening irreversible	dead cells	dead cells

A pressure treatment of 500 atm for 20 hrs rendered a thickening of the cell-wall in both the species. The swelling was relatively greater in the case of *P. urceolata* than *P. nigrescens*. The thickness of the cell-wall in different parts of the thalli as measured immediately after pressure release in both the species are shown in Table 2. The lower part of the thalli exhibited greater swelling than the middle part and the uppermost part showed the least thickening. The cell-walls of the control algae however were less than 0.5 μ in thickness.

TABLE 2. Thickness of the cell wall of *Polysiphonia spp.*, measured immediately after application of hydrostatic pressure at 15°C.

Pressure applied (atm)	Duration of pressure (hrs.)	Part of the thallus	Thickness of the cell-wall (μ)	
			P. urceolata	*P. nigrescens*
500	20	upper	2.7 — 3.4	2.5 — 3.4
		middle	4.5 — 7.2	3.5 — 5.4
		lower	7.2 — 11.7	5.5 — 6.5
800	5	upper	5.4 — 9.0	3.6 — 5.4
		middle	9.0 — 11.0	5.5 — 6.2
		lower	11.0 — 18.0	6.3 — 7.2

A pressure of 800 atm for 5 hrs resulted in still greater thickening of cell-walls in both the algal species. In this case also the swelling observed in *P. urceolata* was relatively greater and irreversible (Fig. 3). On the contrary the thickening produced in the cell-wall of *P. nigrescens* was less intensive and reversible (Fig. 4). When the duration of pressure action was increased to 10 hrs, there was a slight increase in the cell-wall swelling and in both the species the thickening was irreversible. With further increase of pressure duration to 20 hrs the thickening remained more or less the same. The algae in all the cases remained viable.

Pressure level of 1000 atm appeared to be lethal for the survival of *Polysiphonia* (Table 1). After keeping the algae under this pressure for 5 hrs the cells of *P. urceolata* died, while the filaments of *P. nigrescens* exhibited thickened cell-walls with still surviving cells. An increase of pressure duration to 10 hrs was lethal for the thalli of both the species. A further increase of pressure duration to 20 hrs brought the same results in both algal species. The control specimens however showed normal cell-walls.

2. AFTER-EFFECTS OF PRESSURE

The pressure treated algae were regularly examined under the microscope upto 3 weeks' period, and the changes in the cell-wall were compared with those produced immediately after pressure action. In *P. urceolata*, subjected to 400 atm, no effect was

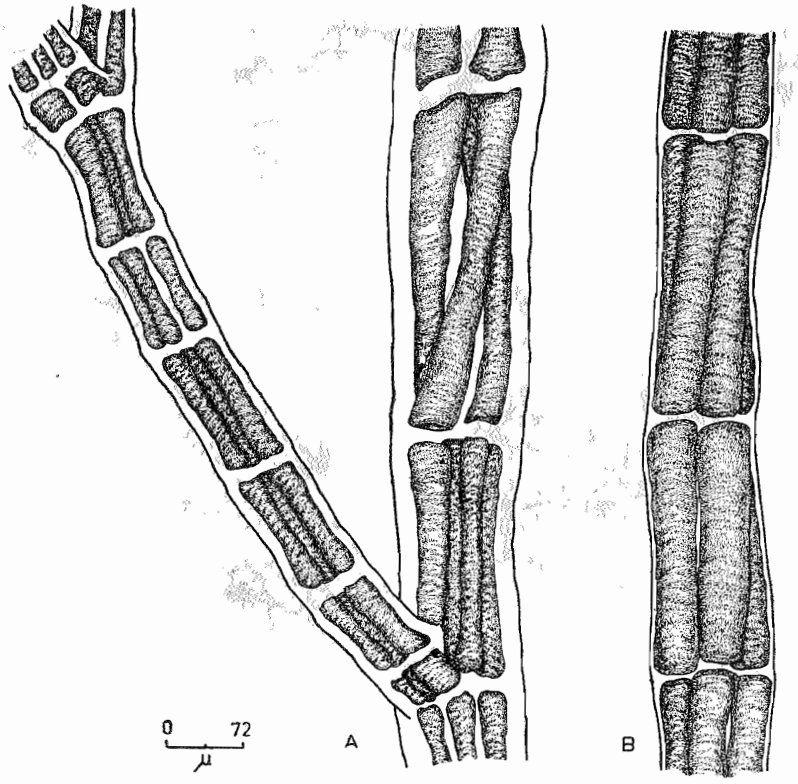

Fig. 3. Part of *Polysiphonia urceolata* filaments, A. swelling produced in the cell-wall as observed immediately after subjection to 800 atm for 5 hs. at 15°C, B. control alga kept under similar conditions but at normal atmospheric pressure.

observed even upto 3 weeks after pressure application. It was interesting to note that a pressure level of 500 atm caused a reversible effect. The black spots and circles produced after a pressure treatment of 500 atm for 5 hrs showed a regular decrease to 30% within 1 week (Table 3). After 2 weeks there was practically no difference between pressure treated and control algae. The algae pressed under 500 atm for 10 hrs as well as 20 hrs also showed a decrease in the pressure effects, and 3 weeks after pressure action it was reduced to 60 and 80% respectively. A pressure of 800 atm on the other hand produced irreversible changes since the algae pressed for 5 hrs died within 3 weeks, those treated for 10 hrs died within 2 weeks and those subjected for 20 hrs died within one week. The control algae however remained unchanged.

P. nigrescens showed similar results, but the pressure effects were less severe than *P. urceolata*. The algae after a pressure treatment of 500 atm for 5 hrs showed no effect on the cell-wall even upto 3 weeks. The black circles and spots seen after a pressure subjection to 500 atm for 10 hrs gradually disappeared (Table 3). They were reduced to 40% within 1 week, and after 2 weeks there was practically no difference between the pressure treated and control algae. The thickening of the cell-wall observed after pressing the algae for 500 atm for 20 hrs also gradually decreased with time, and

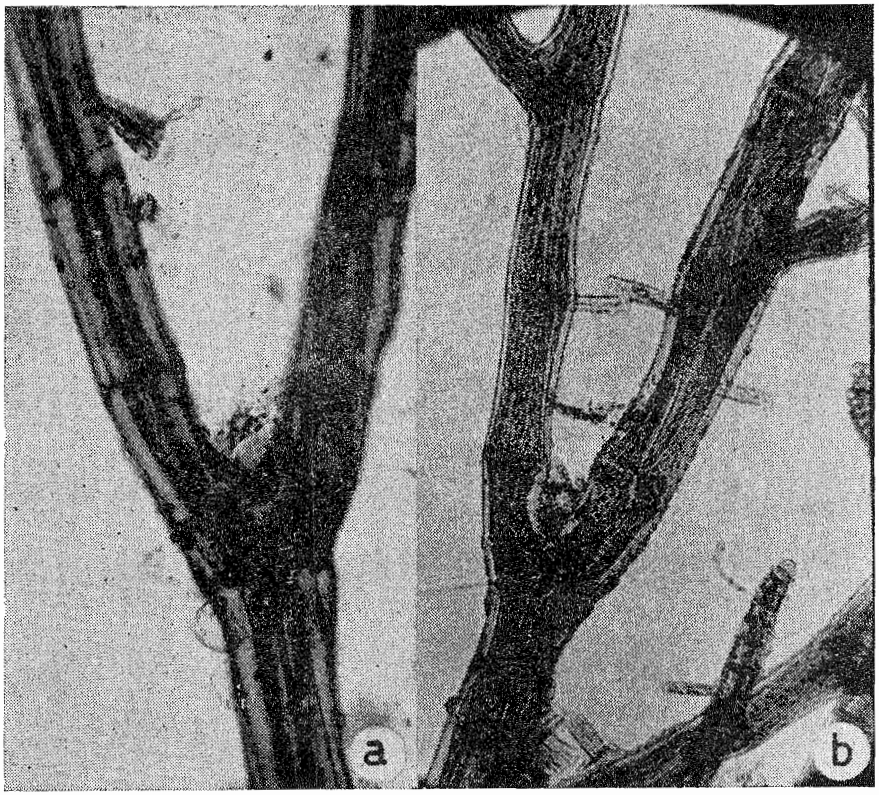

Fig. 4. Filaments of *Polysiphonia nigrescens*, a. control thallus kept at normal atmospheric pressure, b. thickening in the cell-wall as observed immediately after a pressure treatment to 800 atm for 5 hrs at 15°C.

the pressure effects were reduced to 70% after 3 weeks. Similar observations were made after keeping the algae under 800 atm for 5 hrs and the pressure effects were reduced to 90% within 3 weeks.

The thickening of the cell-wall produced by subjecting *P. nigrescens* to 800 atm for 10 hrs was irreversible and the pressure effect was lethal. The swelling remained unaltered and finally led to the death of the algae within 3 weeks. An increase of pressure duration to 20 hrs produced similar results but the severity was increased, and the algae died within 2 weeks after pressure application. A pressure treatment of 1000 atm for 5 hrs which was lethal for the survival of *P. urceolata*, produced irreversible thickening in the cell-walls of *P. nigrescens* and the algae died one week after pressure subjection. The control specimens, however, remained healthy and normal.

Discussion

The above observations present the post-treatment effect of elevated pressures on the cell-wall of two species of *Polysiphonia* collected from different depths. The

TABLE 3. Percentage of changes left in the cell wall of *Polysiphonia spp.* after subjection to hydrostatic pressure at 15°C.

Pressure applied (atm)	Duration of pressure (hrs.)	Days after subjection to pressure						
		0	1	3	5	7	14	21
P. urceolata								
400	20	0	0	0	0	0	0	0
	5	100	95	80	50	30	0	0
500	10	100	100	100	95	90	80	60
	20	100	100	100	100	95	90	80
	5	100	100	100	100	100	100	dead
800	10	100	100	100	100	100	dead	—
	20	100	100	100	100	dead	—	—
P. nigrescens								
	5	0	0	0	0	0	0	0
500	10	100	100	90	70	40	0	0
	20	100	100	100	100	95	85	70
	5	100	100	100	100	100	95	90
800	10	100	100	100	100	100	100	dead
	20	100	100	100	100	100	dead	–
1000	5	100	100	100	100	dead	—	—

shallow-water alga, *P. urceolata* was found to be more pressure sensitive in all the levels of hydrostatic pressure used than *P. nigrescens*, a deep-water representative. Shameel (1973a), while studying the hydrostatic pressure effects on the oxygen gas-exchange of certain marine algae, observed that the shallow-water types were more pressure resistant than the deep-water forms. It was later confirmed by Shameel & Ohno (1972) on some other marine algae. The different behaviour of the species of *Polysiphonia* lies probably in that *P. urceolata* is not a true representative of shallow-water algae, like *Fucus vesiculosus* and *Ulva lactuca* (Shameel, 1973a). Tobler (1909) noticed the occurrence of *P. urceolata* epiphytic on parts of *Laminaria*, where there is an accumulation of organic detritus. As described recently (Pankow, 1971) this alga has a wide ecological range of occurrence in the Baltic Sea. Apart from that there are no true tides in Kieler Förde (Gessner, 1957), from where the algae, studied herein, were collected. The shallow-water algae from this region may although resemble in physiology and behaviour with the intertidal algae, but are not exposed to such extreme temperature fluctuations, direct sunlight and subsequent drying as the intertidal algae have to face during regularly occurring periods of low tide. These algae, unlike their intertidal akins, probably could not develop such adaptive control mechanisms

to protect their enzymes from undergoing denaturation and are thus not so much resistant against pressure stress as they ought to be.

From the taxonomic point of view closely related genera of marine algae, collected from the same locality and habitat, showed genetically fixed differences in the behaviour of their gas exchange under the influence of hydrostatic pressure (Shameel, 1973a). The present study provided an opportunity to observe the behaviour under pressure stress of the two closely related species collected from almost the same area (Kieler Förde). They differed considerably from one another in their resistance to high hydrostatic pressure. This shows that every algal species possesses a characteristic behaviour with regard to its pressure resistance, which is comparable with the behaviour of marine algae in connection with their thermal and osmotic resistance (Schwenke, 1958, 1959). From this it may be concluded that different stimuli e.g., temperature, salinity and hydrostatic pressure have similar action mechanism and like others the pressure resistance may also be considered as genetically fixed physiological characteristic of a particular species. Probably it is due to this reason that different algal species are in possession of different enzyme systems which behave quite differently under the same pressure stress.

The increasing intensity of changes produced in the cell-wall of *Polysiphonia* spp., with the increase of pressure level as well as the duration of pressure action suggests that the hydrostatic pressure does not only bring about the structural changes in the protoplasm but also influences the normal courses of cellular metabolism. The rates of respiration and photosynthesis in several marine algae (Shameel, 1973a) and induction of reproduction and cellular morphology in *Cladophora glomerata* were found to be affected by elevated pressures as well as the duration of their application (Shameel, 1973b). Growth in the cultures of fresh-water algae (Sturm, 1957; Lue-Kim, 1971) as well as fungi were observed to be similarly influenced by pressure (Ahmed & Pritchard, 1970). An increasing injury in the marine animals was noticed with the rising duration of pressure exposure (Ponat, 1967; Naroska, 1968). Due to these similarities it is tempting to speculate that the effects of hydrostatic pressure on marine algae are analogous to those observed in other organisms. The basic life processes of marine algae and other organisms have probably a great deal in common. Fundamentally the pressure intensity and the duration of pressure action must be considered together, both of them collectively constitute the quantity of stimulus.

The pressure level of 500 atm produced changes in the cell-wall of *Polysiphonia* spp., which slowly reverted with time to the normal condition. This probably constitutes the lower limit of tolerance of the algae, because an exposure to lower pressures, brought about no such changes. A subjection to 1000 atm caused immediate death of the treated algae, this appears to mark their upper limit of tolerance. This is in accordance with the observations made by Vidaver (1969), who noticed 3 general types of responses in algae under pressure influences. The first type appeared at about 100 atm, extended upto 700 atm and was imediately reversible upon pressure release; the second one began at 600 atm, persisted to twice this pressure and became slowly reversible with time; and the third type occurred at pressures as low as 1000 atm and was irreversible. The lower and the upper physiological limits of tolerance of the two species, studied herein, differed from each other. The pressure range of the responses mentioned by Vidaver (1969) appear therefore not to be narrowly delineated and there seems to be an appreciable overlapping among them.

The mechanism involved in the swelling of the cell-wall of *Polysiphonia* spp., is difficult to explain on the basis of our present knowledge. As it is the first observation of such type in a marine plant, it appears meaningful to examine the relevant studies made elsewhere. It seems to be comparable to pressure effects on sol-gel transformations. Elevated pressures tend to weaken the more rigid cortical portions of the cell and finally cause solation (Marsland, 1970). Subjection to hydrostatic pressure of cleaving *Arbacia* eggs caused a slow recession untill all the furrows disappeared completely. The occurrence of fine filaments have been observed in the furrow cortex of dividing eggs (Marsland, 1938). It appears to involve processes of depolymeration whereby elongated macromolecular complexes are converted to a number of protein subunits or monomeres. Traditionally high pressure studies have been concerned with physiological activities such as cell-division and movement of cells (Zimmerman, 1970), investigations are needed about the pressure effects on the ultrastructural and molecular levels of the cell. Biochemical studies and the detailed investigations of algal cell-wells are required to elucidate the actual mechanism invovled.

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