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INTERACTIVE EFFECTS OF SALINITY AND NATURAL NITROGEN SOURCE ON SOME METABOLIC ACTIVITIES OF CHLORELLA VULGARIS

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ABSTRACT

Chlorella vulgaris (Chlorophyceae) was cultured on media of combined mixtures containing different salinity levels (ranging from 50-200 mM NaCl) and cow waste extract of concentrations ranging from 0.2-1.4 %. Bold's basal medium was used as a control. The interactive effects of salinity and natural nitrogen source of the test media on the intracellular accumulation of some organic and inorganic solutes were investigated.

The obtained results revealed that the reducing sugars, total free amino acids and free proline content increased under the increasing salinity levels, while they showed a reverse trend with rise in waste concentration. On the other hand, the protein content together with both nitrate and ammonia contents decreased with the increased levels of external salinity. However, increasing waste concentrations were paralleled with remarkable intracellular accumulations of proteins, nitrate and ammonia under an yof the investigated salinity levels.

INTRODUCTION

Salinity represents one of the most important factor which exerts stress injury on the metabolism of plant cells. It was reported that plants growing under conditions of high salinity accumulate various solutes as a result of alterations in intermediary and secondary metabolism of nitrogen or of carbon (Greenway and Munns, 1980; Stewart and Larher, 1980). This results most probably from an imbalance in the inorganic ion status; ultimately causing a malfunctioning of the enzymes involved. Amides, free amino acids, proline, amines, quaternary ammonium compounds and sugars are some of the organic solutes that show a change in their accumulation under conditions of stress (Hsiao, 1973; Stewart and Larher, 1980). It has been suggested that high concentration of organic solutes in the cytoplasm play double role (Greenway and Munns, 1980): (i) they can contribute to the osmatic balance when electrolytes are lower in the cytoplasm than in the vacuole, and (ii) they can have protective effect on enzymes in the presence of

high electrolytes in the cytoplasm. However, there remains speculations about the primary roles of these solutes, viz., whether it is one of storage of reduced carbon and/or nitrogen, or in the osmotic balance of the cell as a whole (Greenway and Munns, 1980).

The interactive effect of salinity and concurrently applied nitrogen and phosphorus fertilizers on the physiology and metabolism of some plants were studied by many workers (Bernstein et al., 1974; Cerda et al., 1977; Selassie and Wagenet, 1981; Wagenet et al., 1980, 1981; Shone and Gale, 1983; Indulkar and More, 1985). However, the mechanisms involved in this interaction are not well understood.

The present work deals with the interactive effects of salinity and the natural nitrogen source in the aquous extract of cow waste on some metabolic activities of Chlorella vulgaris.

MATERIALS AND METHODS

Culturing of algae:

Bold's Basal Medium (Bischof & Bold, 1963) was used as a control medium for culturing of Chlorella vulgaris (Axenic UTEX free-living strain 371).

A series of combined mixtures containing different salinization levels (50, 100, 150, and 200 mM NaCl) and cow waste extract concentrations (0.2, 0.6, 1.0, 1.4 g/100 ml culture) were prepared as described in a previous work (Shaalan et al., 1991). These mixtures were used as culture media for the test organism. Conical flasks of 100 ml capacity, each contained 30 ml culture medium and 2 ml of the axenic alga as inoculum (approximately 0.5 x 10^6 cells /ml culture). All culture experiments were conducted under controlled laboratory conditions with a temperature of $25\pm3^{o}C$, a total light intensity of 4000 lux and a light regime of 16 h light/8h dark.

Harvesting algae preparation of cell-free extract and chemical analyses:

After seven days of culturing and during the exponential phase, Chlorella cells were harvested by centrifugation at 7000 r.p.m for ten minutes. The algal cells were broken down by repeated freezing and thawing using liquid nitrogen. The extraction was completed by grinding with quartz sand in 5 ml of 80% ethyl alcohol. Cell-free extracts were obtained by centrifugation at 7000 r.p.m. for 20 minutes. These extracts were analyzed for the following components by the methods described in their original literature:

- Reducing sugars (Nelson, 1985), Protein content (Hartree, 1972), Total free amino acids (Naguib, 1969), Free proline (Bates et al., 1973)., Nitrate (Johnson & Ulrich, 1950) and Free ammonia (Solorzano, 1969).

RESULTS AND DISCUSSION

The interactive effects of the combined external salinity variations with the different concentrations of cow waste extract on the reducing sugars, proteins, total free amino acids, free proline, nitrate and ammonia contents of Chlorella vulgaris were investigated. The obtaied data are presented in table (1) and Figures (1-6).

The results indicated that the reducing sugars increased with the increase in external salinity levels, but showed a reverse trend with the increase in waste concentrations. However, the percent increases of reducing sugars of the alga grown on the same waste concentrations were more remarkable at high salinity levels than at low ones (Fig. 1).

With the increase of salinity levels from 50 to 200 mM NaCl, the protein content of the rest alga decreased. On the other hand, the different waste concentrations caused an elaborate increase in the algal protein content (Fig. 2). This increase was maximum at the highest waste concentration (1.4%) for all salinity levels applied.

The total amino acids content of Chlorella vulgaris increased under the stress of the all investigated salinity levels (Fig. 3). However, for each salinity levely, the content of total free amino acids decreased with the increase of waste concentration. This decrease was more pronounced at lower salinities than at higher ones.

The content of free proline (Fig. 4) followed the same behavior as the total free amino acids under the same conditions. The maximum value of proline content of the test alga was recorded at salinity 200 mM NaCl with the lowest waste concentration (0.2 %), while the minimum was at salinity 50mM NaCl with the highest waste concentration (1.4%).

Both nitrate and ammonia contents of Chlorella vulgaris (Figs. 5 & 6) decreased gradually with the increase in salinity level. However, at any of the investigated salinity levels, they increased with the increase of waste concentration. The maximum nitrate and ammonia contents were recorded at 50mM NaCl combined with 1.4% waste concentration, while the minimum contents of these inorganic nitrogen forms were at 200mM NaCl with the lowest waste concentration (0.2%). At the highest salinity level, the nitrate content was lower than that of the control.

Although in a previous study (Shaalan et al. 1991); the growth of Chlorella vulgaris was inhibited under the stress of all investigated salinities, yet in the present study a considerable accumulation of some organic solutes was observed.

The increase of reducing sugars, free amino acids and free proline contents may be regarded as an adjustment to

Table 1.

Interactive effects of salinity and natural nitrogen source (cow waste extract) on some organic and inorganic solutes of **Chlorella vulgaris.**

Salinity (mM Na Cl)	50					100				
Waste extract concentration	B*	0.2	0.6	1.0	1.4	B*	0.2	0.6	1.0	1.4
Reducing sugars content (ug /g. d. w.)	21.1	30.8	28.6	26.6	22.9	24.7	38.7	35.9	31.0	29.1
Protein contrent (ug /g. d. w.)	44.5	48.3	52.6	59.1	63.9	40.7	44.1	46.4	48.9	50.3
Total free amino acids (ug /g. d. w.)	7.0	7.1	7.0	7.0	7.0	7.4	8.3	8.1	7.6	7.5
Free proline content (ug /g. d. w.)	0.60	0.73	0.72	0.71	0.66	0.70	0.98	0.94	0.89	0.86
Nitrate content (ug /g. d. w.)	1.87	6.9	7.3	7.7	7.9	2.4	5.4	5.9	6.3	6.6
Ammonia content (ug /g. d. w.)	40.7	44.8	46.3	47.6	50.1	37.4	40.4	41.2	42.2	44.9

* Control (Salinized Bold's Basal Medium without addition of waste).

Table 1 (Cont.)

Salinity (mM Na Cl)	150					200				
Waste extract concentration	B*	0.2	0.6	1.0	1.4	B*	0.2	0.6	1.0	1.4
Reducing sugars content (ug /g. d. w.)	30.4	48.8	49.9	40.2	39.3	36.4	66.4	60.1	51.3	48.7
Protein contrent (ug /g. d. w.)	38.8	39.0	39.6	42.8	46.1	30.9	31.0	31.4	33.0	34.0
Total free amino acids (ug /g. d. w.)	8.1	9.7	9.3	9.0	8.7	8.8	10.8	10.5	9.8	9.7
Free proline content (ug /g. d. w.)	0.83	1.25	1.23	1.19	1.17	1.01	1.7	1.66	1.61	1.56
Nitrate content (ug /g. d. w.)	2.94	3.9	4.1	4.6	4.9	3.4	2.0	2.4	2.8	3.0
Ammonia content (ug /g. d. w.)	31.8	33.6	34.2	35.2	37.4	27.1	27.6	28.5	29.3	29.8

* Control (Salinized Bold's Basal Medium without addition of waste).

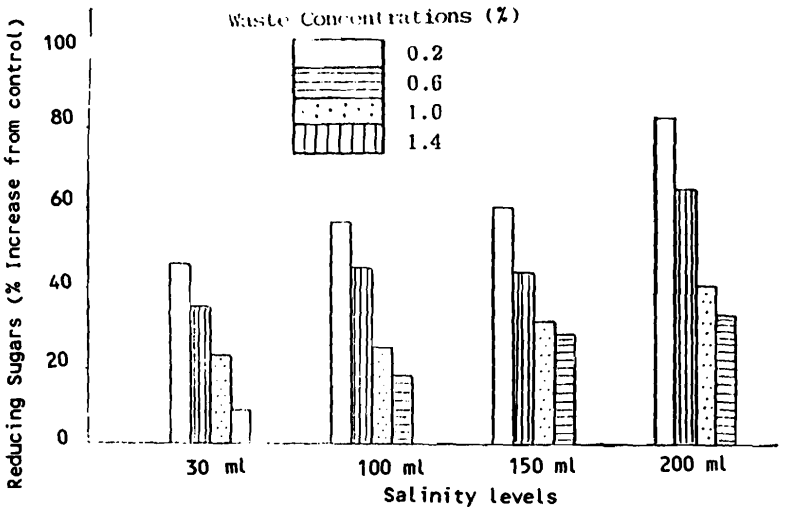

Fig. 1

Interactive effects of NaCl - Waste combinations on Reducing Sugars content of **Chlorella vulgaris.**

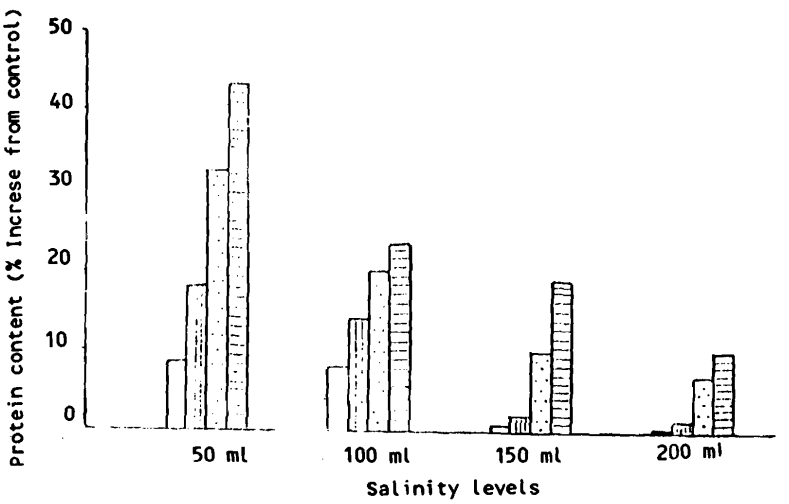

Fig. 2

Interactive effects of NaCl - Waste combinations on Protein content of **Chlorella vulgaris.**

Table 1 (Cont.)

Salinity (mM Na Cl)	150					200				
Waste extract concentration	B*	0.2	0.6	1.0	1.4	B*	0.2	0.6	1.0	1.4
Reducing sugars content (ug /g. d. w.)	30.4	48.8	49.9	40.2	39.3	36.4	66.4	60.1	51.3	48.7
Protein contrent (ug /g. d. w.)	38.8	39.0	39.6	42.8	46.1	30.9	31.0	31.4	33.0	34.0
Total free amino acids (ug /g. d. w.)	8.1	9.7	9.3	9.0	8.7	8.8	10.8	10.5	9.8	9.7
Free proline content (ug /g. d. w.)	0.83	1.25	1.23	1.19	1.17	1.01	1.7	1.66	1.61	1.56
Nitrate content (ug /g. d. w.)	2.94	3.9	4.1	4.6	4.9	3.4	2.0	2.4	2.8	3.0
Ammonia content (ug /g. d. w.)	31.8	33.6	34.2	35.2	37.4	27.1	27.6	28.5	29.3	29.8

* Control (Salinized Bold's Basal Medium without addition of waste).

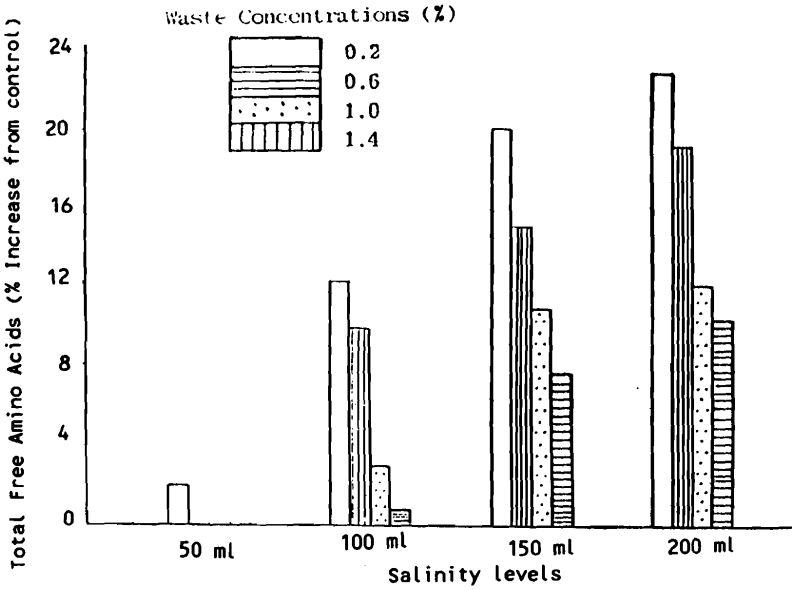

Fig. 3

Interactive effects of NaCl - Waste combinations on Total Free Amino Acids of **Chlorella vulgaris.**

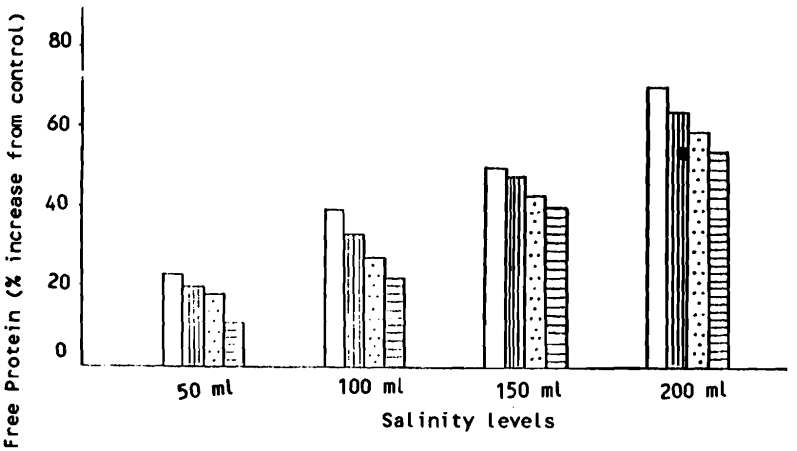

Fig. 4

Interactive effects of NaCl - Waste combinations on Free Proline content of **Chlorella vulgaris.**

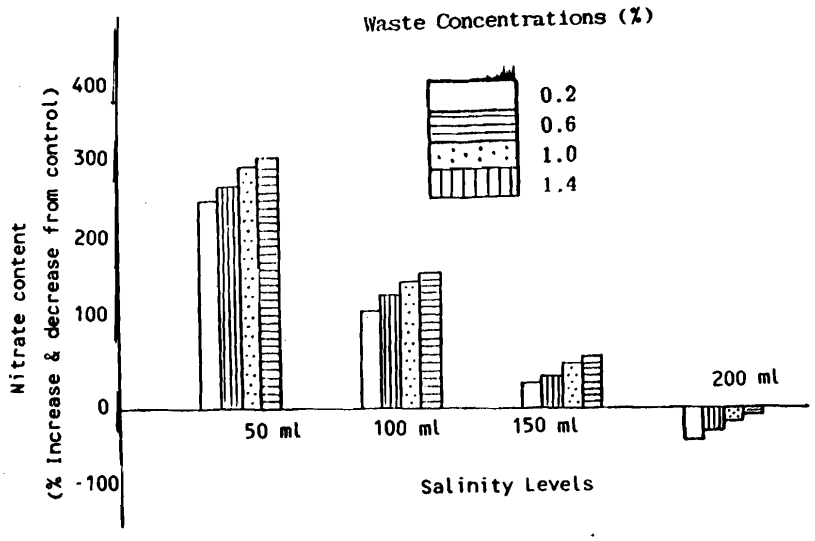

Fig. 5

Interactive effects of NaCl - Waste combinations
on Nitrate content of **Chlorella vulgaris.**

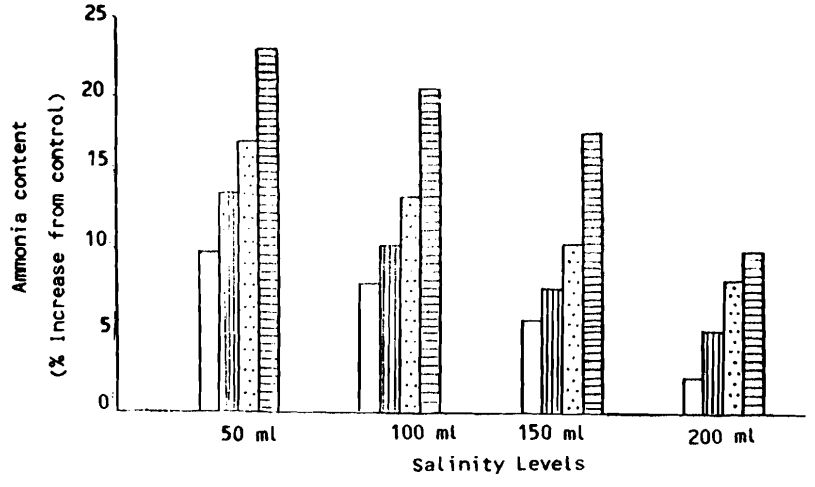

Fig. 6

Interactive effects of NaCl - Waste combinations
on Ammonia contents of **Chlorella vulgaris.**

the unbalanced osmoregulation and /or disturbed metabolism under the stress conditions (Stewart & Hanson, 1980 and Imamul Huq & Larber, 1983). It is thought that the adaptive role of proline is related to the algal survival rather than to the process of its growth (Greenway & Munns, 1980).

In the present studt, the protein content of Chlorella vulgaris decreased sharply with the increase of salinization levels, but showed a reverse trend with the increase of waste concentration. This indicated that although the algal growth was negatively affected by the external salt, the N-status and the growth of the alga were apparently enhanced by the addition of the nitrogenous compounds from the natural source. In fact, growth reduction under salt stress was suggested to have resulted from "ion excess" (Imamul Huq & Larber, 1983).

The obvious cellular decreases in both nitrate and ammonia contents of Chlorella were parallel to the increase in external salinty levels. Similar observations were reported for **Porphyridium** and **Cyclotella** cultured under salt stress (Dickson & Kirst, 1087). However, the intracellular accumulation of both inorganic nitrogen forms by Chlorella **vulgaris may be due to their increased uptake from the outside medium which increased with the increase of waste concentration especially under low salinities.**

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