

**ROLE OF SULPHUR SOURCES
IN GROWTH AND METABOLISM
OF PHORMIDIUM ANGUSTISSIMUM**

THESIS

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

« وما أوتيتم من العلم إلا قليلاً »

صدق الله العظيم

هدية مقدمة من :

التاريخ / /

This GIFT was donated by :

Prof. Mohammed E. NAIJIB

Date 23-6-1985

DEDICATION

To my hope

To my love

**I devote my life & my
thesis to her**

To my daughter.....DAHLIA

A C K N O W L E D G E M E N T S

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This dissertation has not previously been submitted for a degree at this or at any other university.

The references in the text will show specifically the extent to which I have availed myself of the work of other authors.

Sekina Tawfik

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PART I

INTRODUCTION

A- HISTORICAL REVIEW

The continuous production of air pollutants represents a serious environmental problem. Sulphur emissions comprise one of the major air contaminants during the last twenty years. The quantity of sulphur, in nature, have increased several times as a result of increased utilization of sulphur containing materials that led to rapid mobilization, transportation and subsequent deposition and accumulation of sulphur in natural ecosystems.

Sulphur dioxide is one of the most frequently occurring air-polluting substances. Sulphur dioxide is released in the greatest quantities due to the increasing use of fossil fuels to generate electricity. Other significant contributions come from industrial processes such as refining and smelting (Robinson and Robbins, 1972; Friend, 1973). It is also widely used in food preservation as antimicrobial agent and as antioxidant. Compounds of sulphur, found in the atmosphere, include sulphur dioxide, sulphur trioxide, sulphate containing compounds viz. sulphuric acid and ammonium sulphate, hydrogen sulphide, methyl mercaptan, dimethyl sulphide, dimethyldisulphide and other organic forms.

Oxidation of sulphur dioxide leads to the formation of sulphate. Sulphur trioxide is emitted with sulphur dioxide in very small amounts. It combines very rapidly with water to form sulphuric acid.

Sulphate containing compounds are the end product of atmospheric oxidation reactions of sulphur dioxide. They may exist as components of the atmosphere, as solid or liquid particles. On contact with water or the surface of wet soil particles, sulphur dioxide readily enters solution and dissociates.

The percentage of the major anionic products, likely to be encountered under most environmental circumstances, is given by Babich and Stotzky (1978).

Hydrogen sulphide is formed primarily from the natural decay of vegetation, marsh lands and in oceans. In recent marine sediments, rich in organic matter, the major process affecting sulphur geochemistry is bacterial reduction of the sulphate ion, producing dissolved sulphide. Dissolved sulphide subsequently reacts with iron, resulting in a metastable intermediate iron sulphide, and eventually sedimentary iron pyrite (Kaplan et al., 1963; Rickard, 1969; Love, 1969; Berner, 1970; Sweeney,

1972; Goldhaber and Kaplan; 1974; Jorgensen and Fenshel, 1974). In air, hydrogen sulphide is probably oxidized to sulphur dioxide within hours.

Relatively little is known about sulphur containing organic compounds. They are primarily from natural sources, and emissions of dimethyl sulphide along with hydrogen sulphide may be important in global sulphur budget. (Granat et al., 1976).

Microbes are the primary source of many gaseous atmospheric pollutants, including hydrogen sulphide and organic sulphur containing volatiles, such as methyl mercaptan, dimethyl disulphide, dimethyl sulphide. Gaseous emissions, from microbes, in combination with those from other biologic and abiotic sources, constitute the natural background levels to which are added emissions from anthropogenic sources.

Sulphur compounds in the atmosphere are mainly (a) sulphur dioxide, primarily from anthropogenic sources, although small amounts of the gas are also released from volcanoes and (b) hydrogen sulphide, primarily from anaerobic microbial reductions of sulphate and/or aerobic microbial decomposition of sulphydryl-containing amino acids (Babich and Stotzky, 1978).

In addition, microbial decomposition of organic matter, in soil, results in the evolution of a variety of reduced sulphur containing compounds such as methyl mercaptan, dimethyl sulphide and dimethyl disulphide (Babich and Stotzky, 1972, 1974; Rasmussen, et al., 1975; Stotzky and Schenek, 1976).

Gaseous sulphur compounds undergo a variety of catalytic and photochemical reactions, in the atmosphere, with the subsequent formation of secondary products. Atmospheric sulphate salts are formed from dimethyl sulphide or hydrogen sulphide albeit by different processes (Babich and Stotzky, 1972, 1974).

Sulphate is the sole component forming a minor portion of the total sulphur content of soils. The bulk of soil sulphur is in the organic form which is metabolized by soil microorganisms to render it available, in an inorganic state, for plant nutrition.

Sulphur is bound in proteins, of vegetable and animal origin, and in protoplasm of microorganisms, in the form of sulphur containing amino acids (cystiene and methionine) and B-vitamins (Subba, 1977). The conversion of organically bound sulphur to the inorganic state

is known as mineralization of sulphur and is mediated through microorganisms. The sulphur thus released, is either absorbed by plants or escapes to the atmosphere in the form of oxides.

In absence of oxygen, certain microorganisms produce hydrogen sulphide from organic sulphur substrates especially in water logged soils (Alexander, 1961). Oxidation and reduction of sulphur involve both organic and inorganic compounds. Rapid and striking changes in soil inorganic sulphur compounds are brought by such microorganisms. Several microorganisms of wide natural distribution are able to oxidize thiosulphate, sulphide, and elemental sulphur, when grown in almost neutral media (Starkey, 1966; Landesman, et al., 1966). Among these microorganisms Thiobacillus deserves special mention as it produces sulphuric acid when elemental sulphur is added to soil leading to a drop in soil pH to 2, after prolonged incubation with the bacterium (Subba, 1977).

A wide range of heterotrophic organisms including bacteria and fungi, are able to oxidize both elemental sulphur and thiosulphate (Kelly, 1968). Sulphur oxidizers

were isolated from polluted leaves, litters and soils (Wainwright, 1978). Contaminated soils yield nearly one hundred times more colonies than unpolluted soils.

Wainwright (1979), reported that soils exposed to atmospheric pollution($125 \text{ SO}_2 \text{ ug m}^{-3}$) contained significantly greater numbers of Thiobacilli and sulphur oxidizing fungi than did similar but unpolluted soils.

The oxidation of elemental sulphur by Thiobacillus denitrificans requires an enzyme present in the S_{144} fraction GSH as well as a heat-stable factor (Aminuoddin, 1979).

Yeasts are also able to oxidise sulphur compounds. Kurek (1979), found that Rhodotroula, is able to oxidize elemental sulphur and thiosulphate in vivo and thiosulphate and sulphite in vitro. He reported that the enzymes involved in the oxidation of thiosulphate and sulphite are non-inducible.

Subba (1977), concluded that the various transformations of sulphur, in the biosphere, can be summed up as a cyclic reaction involving (1) decomposition of organic sulphur compounds into subunits which are, in turn,

converted into organic compounds through a process of mineralization, (2) assimilation of sulphur into the protoplasm of microorganisms, a process referred to as immobilization, (3) Oxidation of inorganic sulphur compounds into elemental sulphur, (4) reduction of sulphate.

Natalia et al. (1977) proved the applicability of microbiological tests for determination of the degree of soil pollution with toxic factors present in dusts emitted by copper smelters, containing sulphur oxides.

The toxicity of sulphur dioxide is a function of its high solubility in water and consequently, to its solubility products and, subsequent to their oxidation products. Sulphur dioxide, and droplets of sulphuric acid, in the air affect animal and people. Sulphur dioxide is absorbed from the respiratory tract into the blood stream and distributed through the body (Balchum et al., 1960 a, b; Yokoyama et al., 1971), where it appears to be metabolized and excreted via the urinary tract. Relation between day-to-day changes in mortality and pollution has also been reported from Osaka (Watanabe 1966). There appeared to be increases in mortality

(about 20 %) on days associated with the high levels of sulphur dioxide. Buechley et al. (1973) found more or less similar results.

Most studies with sulphur dioxide were focussed on lichens, bryophytes, and crops of agricultural importance either as monitors of air pollution level or for economic reasons. Babich and Stotzky (1974), reviewed the available data on the relationship between atmospheric contaminants and the soil ecosystem. Eloranta and Pertti (1980), studied algal growth in water receiving sulphite and sulphate cellulose effluents. They found that, at high concentration of sulphite and pH 4, algal growth was almost totally arrested. The inhibition decreased with increasing dilution of effluents (increasing distance from factories).

Gusev et al. (1979) noticed that cyanobacteria belonging to the genera Oscillatoria and Anabaena, could grow in water reservoirs with high content of sulphides (up to 9 mM).

Again, Monheimer (1979) found that up to 500 ug sulphur/L of an equimolecular mixture of cysteine and methionine decreased the rate of sulphate uptake by Ankistrodesmus

falcatus without affecting that by Navicula pelliculosa. In the meantime, 50 ug. S/L inhibited sulphate uptake by Anabaena flos-aquae.

The same author reported that, in most fresh water systems, the effects of organic sulphur pollution on algal sulphate uptake and primary productivity are insignificant compared to other ecologic changes resulting from such pollution. Tuerk and Renate (1978) found that the sulphur content of the lichen thalli increased with higher levels of sulphur dioxide-pollution. Spedding et al. (1980), studied the effect of pH on the uptake of S^{35} labelled sulphate, sulphite and sulphide by Chlorella vulgaris. They found that with sulphate, an enhanced uptake; in the cell, at pH 5.0 occurred. This was attributed to transport of $SO_2 \cdot H_2O$. The uptake of sulphide by Chlorella vulgaris was strongly pH dependent, with high uptake at low pH. They also, found that the damaging effects of sulphite solutions on growth and chlorophyll contents of Chlorella vulgaris were directly related to concentration of $SO_2 \cdot H_2O$, whose toxicity levels were in the n mole/L range. The solution pH is an important factor for the toxicity levels of sulphite and sulphide.

Sulphur dioxide affects plants in several ways. Ichikawa et al. (1975), found that exposure of kidney beans, in pot experiments, to sulphur dioxide, reduced the dry weight of the plant. Also Singh (1979), found that addition of sulphur to soil up to 80 ppm increased growth and grain yield of soy bean.

Higher applications (140 ppm) significantly decreased the yield. Jaeger (1977), noticed that exposure of spruce and pea plants to sulphur dioxide increased their glutamate as well as the amino acids of the glutamate family, coupled with increased activities of glutamate dehydrogenase and glutamine synthetase, whereas, glutamic-pyruvate-transaminase and glutamic-oxalo-acetate transaminase were inactivated.

Kumar and Singh (1980), found that the application of sulphur up to 80 ppm significantly increased dry matter and grain yield in soybeans.

Sprugel et al. (1980) carried out experiments to identify injuries to soybean plants exposed to elevated levels of sulphur dioxide (0.09 - 0.79 ppm) during fumigation. Although visible injury was observed in only 2 plots, yield, at harvest, was reduced in every fumigated

plot compared to nearby unfumigated control plots.

Ferguson et al. (1978), working with Sphagnum species, found that the species differed in their response to the pollutant. Bressan et al. (1978), attributed the principle reason for special and varietal differences in resistance, among different cultivars, to the relative rate of absorption of gas.

Jaco (1977), working with rice plants found that the sensitivity diminished with age. Smith and Davis (1977), found that the secondary pine needles were generally more sensitive to sulphur dioxide than the primary ones. The cotyledons were generally more tolerant than needles.

Laurence (1979) noticed that maize hybrids were more tolerant to low sulphur dioxide doses (0.1-0.6 ppm.) than wheat. Still difference in sensitivities among wheat cultivars, were observed.

Not only sulphur dioxide caused visible changes in the plant but also affected chloroplasts, photosynthesis and some other chemical activities.

In this connection, the work of Markowski and Schramel (1975) may be mentioned. They found that under

optimum soil moisture conditions, sulphur dioxide produced a significant drop in dry weight and reduced plant height. They also found that chlorophyll content was either lower (sunflower, barley, maize) or higher (tobacco, beans) than control plants. Hallgren and Huss (1975), found that sulphur dioxide inhibited photosynthesis and nitrogen fixation by blue green algae.

Ushijima et al. (1976), found that sulphur dioxide treatment reduced carbon dioxide intake by sunflower plants. The reduction of photosynthesis and transpiration started very rapidly. However the inhibitory ratio became constant thereafter. Their results showed also that sulphur dioxide seemed to directly inhibit carbon dioxide fixing system.

Black and Unsworth (1979), reported lower photosynthetic activities in Vicia faba exposed to sulphur dioxide polluted air. Hirai and Tsubota (1975), found that chlorophyll a was apt to be degraded than chlorophyll b.

When circular discs, cut from roadside tree leaves, were soaked in 0.01 M sodium sulphite, for 24 hours at 25°C, and illumination intensity of 3500 lux., the degree of degradation differed with plant species.

Spedding et al. (1980b) emphasised the importance of pH of the medium for toxicity levels of sulphur dioxide and hydrogen sulphide to isolated chloroplasts. They found that sulphate showed a constant uptake rate of about $3.6 \text{ } \mu\text{mol } \mu\text{l}^{-1} \text{ stroma space min}^{-1}$ within the whole pH range (5-8). Sulphite gave an uptake rate of a similar value at higher pH values; which increased as pH decreased to pH 5. Sulphide showed uptake rates ranging from $10 \text{ n mol } \mu\text{l}^{-1} \text{ stroma space min}^{-1}$ at pH 7.5 to $35 \text{ n mol } \mu\text{l}^{-1} \text{ stroma space min}^{-1}$ at pH 4.9. They attributed the increasing uptake of sulphite and sulphide with decreasing pH, to the simple diffusion of SO_2 , H_2O and H_2S (aq) respectively, across the inner chloroplast membrane. They also noticed that, with all sulphur compounds investigated, an increase in uptake with increase of light intensity was found, whereas the earlier light-triggered uptake of sulphate and sulphite was found to be related to ATP and to carrier-SH group supply respectively.

On the other hand, Mourioux and Roland (1979), reported that the rate of transport for sulphate through the envelope of spinach chloroplast was not influenced

either by illumination or pH change in the external medium. They also found that phosphate was a competitive inhibitor of sulphate uptake by chloroplasts. The rate of transport for phosphate appeared to be much higher than for sulphate and when the chloroplasts were preloaded with labelled sulphate, radioactivity was rapidly released after addition of phosphate into the external medium.

Again Hampp et al. (1980) preloaded isolated spinach chloroplasts with labeled sulphite or sulphate and washed them before the efflux of label into the suspension medium measured. The results revealed that sulphate and sulphite, in the medium, enhanced the rate of efflux, indicating that a facilitated back-exchange diffusion is involved. They also found that the efflux of label was accelerated by illumination. The observed stimulation of transport by light was obviously due to change in competition for transport between inorganic phosphate and inorganic sulphur in the course of light/dark transitions and not due to inorganic sulphur transport activated by an envelope associated ATP-ase.

An experiment, carried out by Shimazaki and Kiyoshi (1980), on the inhibition site of the electron transport system in lettuce chloroplasts isolated from sulphur dioxide fumigated leaves at 2.0 ppm, showed inhibition in electron flow from water to 2,6-dichloroindophenol (DCIP); but the electron flow from reduced DCIP to methyl viologen was not affected. Neither diphenylcarbazine nor manganese chloride could restore the activity of DCIP-Hill reaction of sulphur dioxide injured chloroplasts. They also reported that the pool size of the primary electron acceptors decreased with sulphur dioxide fumigation. The gas inactivated the primary electron donor or the reaction center itself.

Sulphur and sulphur compounds affect the morphological characteristics, sporulation, biochemical and biological activities as well as pathogenicity of microorganisms. Many investigators proved that air pollutants have the potential to decrease or increase the resistance of the host organism to infection (Saunders, 1971; Heagle 1973; Babich and Stotzky, 1974).

Sulphur dioxide has been shown to inhibit growth and respiration of bacteria, fungi, algae and lichens;

to retard germination of fungal spores, to decrease survival of air borne bacteria and animal viruses, and to inhibit nitrification rates in soils (Bozian and Stotzky, 1976). Bisulphite, a product formed in water from sulphur dioxide, may also inhibit other microbial groups (Wodzinski et al., 1978).

Glucose oxidation was inhibited in a forest soil (pH 4.01) previously exposed to 1.0 ul of sulphur dioxide per liter. The extent of inhibition and the decline in pH being directly related to the length of exposure (Grant et al., 1979). Mahmoud et al. (1977), found that addition of potassium sulphate to water logged clay-loam soil increased the total counts of microbial flora over the control. On the other hand, Mejstrik (1978) indicated that 84 or 168 $\mu\text{g}/\text{m}^3$ sulphur dioxide were injurious to Flammulina velutipes, Nematoloma fasciculare and Pleurotus ostreatus. The latter dose arrested growth of the three organisms without affecting the morphological structure of the hyphae. Schimz (1980) found that after a short period of tolerance, living cells of Saccharomyces cerevisiae were irreversibly damaged by small sulphite doses. The length of tolerance period and the

rate of damaging effect depended on sulphite concentration, pH value, temperature, the physiological state of the cells and incubation period.

Mejstrik (1978), found that fumigation of three fungal species, with different concentrations of sulphur dioxide inhibited their growth. The inhibitory effect increased with increasing sulphur dioxide concentration 1.68 ug/m^3 of the gas arrested growth of the three species.

Sulphur compounds have certain effects on living cells. Matsushima et al. (1975) reported that sulphur dioxide-treated leaf cells clearly showed severe plasmolysis with destructed ribosome and cytoplasm. Also Stoeckli et al. (1975), found that sulphur dioxide led to considerable shrinkage of the cell nuclei in the apical meristem. Soldatini et al. (1978) found that higher concentrations of sulphite increasingly inhibited cell growth.

Comparative studies by Haertel and Sieglinde (1974) showed that sulphurous acid was much more toxic than sulphuric acid within plant cells. It destroyed the protoplasm 2.5 to 20 times more rapidly than sulphuric acid.

Glasshouse experiments by Masohara and ~~Kaneo~~ (1974) showed that continuous exposure of chard, spinach, Chinese cabbage and Japanese radish to 0.4 ppm sulphur dioxide for one week induced peripheral and intervenal necrosis followed by dehydration.

Ziegler (1978), working with Chlorella vulgaris concluded that cell growth inhibition increased with sulphite concentration; the response to sulphite ion was confined to the very beginning of culture growth.

Sulphur dioxide affects also various metabolic processes. Zeigler (1973), showed that, in contrast to ribulose diphosphate carboxylase, phosphoenolpyruvate carboxylase of corn leaf extracts was not inhibited by 10 millimoles sulphite ion. Furthermore although malate dehydrogenase was almost 85 % inhibited, at this sulphite dose yet malate formation was unaffected.

Haertel and Sieglinde (1974) found that sulphur dioxide affected permeability, osmotic values and plasma flow at a sulphurous acid concentration of 0.00001 N while starch metabolism was greatly accelerated. The experiments also indicated that sulphurous acid first attacked the osmotic system of the cell by interfering with cellular-SH groups.

Mayumi and Yamazoe (1971), working on the affect of sulphur on protein metabolism, showed that 10 % of the absorbed sulphur dioxide was assimilated into protein and the remainder was detected in the water soluble forms. Khvoles and Korobko (1977) found that dimethyl sulphate caused sharp increase in the RNA content in the nuclei of loach females. Harvey and Allan (1979), working with Pinus contorta, showed that ATP content of needle cells declined as sulphur dioxide concentration increased in the ambient air. Ziegler (1972) showed that sulphite anion showed a non-competitive inhibition patterns with respect to ribulose 1:5 diphosphate carboxylase and Mg ion, as well as a competitive inhibition pattern with respect to bicarbonate anion. Shapiro (1977), working on the genetic effect of bisulphite, observed inhibition of DNA synthesis, cell division and mitosis, accompanied by chromosome aberrations or mutations. This has been demonstrated in microorganisms, plants and mammalian cells.

Hanson et al. (1973) showed that readily available sulphur sources, such as sulphate or methionine, repress the synthesis of the proteolytic activity by Neurospora crassa. Grant et al. (1979) found that bacterial number

in soil, incubated for 48 hours with 0.1 ul of sulphur dioxide/L, were reduced, but the bacteria grew rapidly if glucose or an amino acid mixture was added after the exposure period. Their results further showed that low levels of bisulphite inhibited amino acid decomposition in soil at pH 3.8 but the effect was less pronounced at pH 4.0. Malhotra and Khan (1980) reported that treatment of pine seedlings with sulphur dioxide inhibited the acid phosphatase activity after exposure of the seedlings to 0.35 ppm. concentration for 24 hours, the inhibition was more pronounced in the older (3 months) than the younger (1 month) tissues from the same seedlings. Upon removal of plants from sulphur dioxide environment the inhibitory effects of fumigation were completely reversed in plants exposed to short duration (1 hour), but were unchanged in plants exposed for a long duration (24 hours).

Sakaguchi et al. (1972) showed that nitrate reductase by washed cells of E. coli was accelerated by the presence of sulphite in the reaction medium. Yokota et al. (1972), found that the hydrolytic enzymes of Aspergillus

fumigatus and Bacillus subtilis were not affected by sulphur oxides.

Kommunarskaya (1970), studied the lethal and mutagenic effects of S^{35} on Penicillium nigricans, producing griseofulvin. He noticed that, in Czapek medium with S^{35} , all stable sulphur was replaced by the radioactive form in the form of $Na_2SO_4^{35}$. The sulphur isotope increased the morphological and antibiotic reactions. A new variant with high levels of griseofulvin production (by 60 to 70 %) was selected. The new strain differed from the initial by its morphological and cultural properties.

B- MATERIAL AND METHODS

B- MATERIAL AND METHODS

The organism used in this investigation, Phormidium angustissimum, was isolated from the Egyptian garden soil of the Cairo University. It was kindly identified by Prof. W. Koch; Sammlung von Algen Kulturen, Pflanzenphysiologie, Institut der Universität Göttingen, to whom the author is greatly indebted and thankful.

The finely powdered soil samples were spread, in 1 cm thick layer, on the equator of a sterile agar plate (El-Ayouty, 1966) and periodically moistened with sterile distilled water while standing in diffused light. Small colonies of the blue green algae radiate from the soil border. These were aseptically removed and homogenized with little sterile distilled water. Using a micro-atomizer, the homogenate was sprayed over nutrient agar plate, kept in indirect light. The newly appearing colonies were examined for purity and re-transferred to new solid or liquid media.

Parallel with such procedure other colonies were aseptically smeared in little sterile distilled water. By the help of a micropipette and microscopic examination, single filaments were aseptically picked out of the

mixture, washed in sterile distilled water and passed into a sterile culture solution. These were left to grow then re-examined for purity.

The obtained uni-algal culture was freed of bacteria applying a combination of Pringsheim (1951) and Pinter and Provasoli (1958) methods. 1 ml concentrated algal suspension, mixed with 150 units penicillin and 3 ug dihydrostreptomycin was inserted in a well formed in the center of a 4- mm thick, sterile silica gel plate, moistened with a nutrient solution. This antibiotic mixture was found to suppress the bacterial growth, allowing the filaments to grow rapidly. Several transferrences from the outer zones to the same concentrations of antibiotics were necessary to establish a bacteria-free culture. Finally a block of silica gel, with filaments from the outer zone was inverted on a nutrient-agar plate devoid of antibiotics.

In order to ensure complete absence of bacteria, an algal inoculum was inserted on either Dox-agar; peptone-dextrose agar or yeast-dextrose agar slants and kept for 3 weeks, in the dark, at 35°C, whereby no bacterial

growth was noticed on the surface while the alga was bleached.

Furthermore, all experiments were carried out under florescent light which produced mild ultra violet irradiation that helped, to a certain extent, in the sterilization of the algal cultures.

The nutrient solution, used by Shakeeb (1975) in his Ph.D. thesis was used in this investigation. It has the following composition:

Potassium nitrate	1.50 gm
Dipotassium hydrogen phosphate	0.50 gm
Magnesium sulphate	0.20 gm
Sodium bicarbonate	1.80 gm
Sodium chloride	1.00 gm
Calcium chloride crystals	53.00 mgm
Manganese chloride	1.81 mgm
Copper sulphate	0.08 mgm
Cobalt chloride	0.044mgm
Zinc sulphate	0.22 mgm
Boric acid	2.86 mgm
Ammonium molybdate	0.015mgm
Nickel sulphate	0.047mgm
Sodium tungstate	0.179mgm
Potassium alum	0.096mgm

All in one litre to which was added 3 ml of 0.1 M EDTA solution.

Method of Experimentation:

Stock cultures of the organism were prepared by inoculating 200 mls of the medium in 250 ml flasks. Several inoculated flasks were aerated with sterile air, free of combined nitrogen. This is accomplished by following the procedures adopted by Fogg(1942). The slow air current (3 liters per hour per flask) was passed in half-filled 2-litre-capacity jars, with sintered glass bubblers, containing 5 % copper sulphate, followed by 1 % sodium carbonate, 2 % mercuric chloride then 25 % sulphuric acid before passing in a 30 cm length of sterile cotton glass cylinder. Before passing to the culture solutions, the air passed through another bottle containing sterile bidistilled water, then another empty bottle to avoid the back suction.

10 days old cultures were combined, homogenized in the automix (4000 r.p.m) for 5 minutes and diluted twice. Under aseptic conditions, 5 ml diluted mixture was transferred to 195 ml of the experimental media. All flasks were incubated in a large air incubator at $28^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ under continuous light (4000 lux).

At the expiry of the experimental period, the culture mass was separated from its medium by suction filtration. The alga was rinsed with little water. Both filtrate and washings were combined and made to 200 ml by using bidistilled water. The filtered media were kept in narrow mouthed containers, covered with toluene, in the refrigerator for analysis.

The algal masses were further thoroughly washed and dried by further suction. 5 replicates, from each treatment, were directly put in a hot air dry oven, at 90°C for 30 minutes then transferred to 50°C until a constant weight was attained. Another set was homogenized with 10 ml borate buffer, at pH 8 before centrifugation while a third set of 5 samples was extracted with 90 % acetone for chlorophyll estimation.

Pigment Estimation:

The acetone extract was diluted to the appropriate concentration before its colour intensity was measured at 663, 647 and 452 nm. (Metzner et al., 1965). The following equations were applied in calculation of the pigment constituents of the experimental algae in mg per litre.

Chlorophylla = $11.78 \text{ E } 663 - 2.29 \text{ E } 647$

Chlorophyll b = $20.05 \text{ E } 647 - 4.77 \text{ E } 663$

Carotenes = $4.75 \text{ E } 452 - (\text{Chl. a} + \text{Chl. b}) \times 0.226$.

CHEMICAL ANALYSIS

In this investigation, estimation of nitrogen and phosphorus components was performed using the available colorimetric procedures, at hand.

A- ESTIMATION OF SOLUBLE MATTER:

1. Ammonia Nitrogen:

The procedures mentioned by Fawcett and Scott (1960) and Chaney and Marbach (1962) were followed in this investigation. 1 ml of 5 % phenol containing 25 mgm sodium nitroprusside, was added to a known volume of the neutral extract followed by 1 ml of 2 % sodium hypochlorite dissolved in 0.63 N sodium hydroxide. The mixture was incubated, at 37°C, for 15 minutes, diluted to the appropriate volume and the optical density determined at 630 nm.

2. Nitrate nitrogen:

According to Paech and Tracey (1956), in absence of interfering chloride ions, nitrate could be estimated by evaporation of a known aliquot of the medium or fungus

extract, on a boiling water bath till dryness then left to cool, 1 ml phenoldisulphonic acid was added and shaken. It was kept for 10 - 15 minutes before addition of few ml distilled water followed by 20 % sodium hydroxide till the mixture is alkaline. The mixture is made up to a known volume with distilled water and the intensity of the developed yellow colour was measured photometrically at 430 nm.

In this investigation, comparison of the results, obtained by this method; with those obtained by the silver sulphate method (Paech and Tracey, 1956) revealed no significant difference.

3- Nitrite Nitrogen:

The diazotiazation with sulphanilic acid followed by coupling with α -naphylamine in acetic acid is the basic principle for the colorimetric estimation of nitrite nitrogen (c.f. Paech and Tracey, 1956) at 530 nm.

4- Free Amino Nitrogen:

A protein-free sample was made slightly alkaline to phenol phthalein before addition of 1 ml of 1 % borax (Russel, 1944). After shaking, another 1 ml of 0.25 % freshly prepared naphthoquinone-4-sulphonic acid was

added. The mixture was kept in boiling water bath, for 15 minutes, then rapidly cooled. Acid formaline and thiosulphate solutions were added and shaken. The mixture stood 20 minutes before estimation of its colour intensity at 470 nm.

5- Peptide Nitrogen:

The procedures of Lowry et al. (1951) were followed. A sample of the borate extract was mixed with 1 ml freshly mixed (1:1 ratio) solution of 2 % sodium carbonate in 4 % sodium hydroxide and 0.5 % copper sulphate in 1 % sodium tartarate. The mixture stood for 10 minutes before addition of 0.1 ml Folin phenol and made up to volume.

The optical density of the mixture was measured, after 30 minutes, at 700 nm.

6- Other Soluble Nitrogen:

If we subtract the product of peptide, amino and ammonia nitrogen from the value, obtained experimentally, for total soluble nitrogen, we can calculate the value of other soluble nitrogen including amide and acyclic nitrogen (purines and pyrimidines).

7- Total Soluble Nitrogen:

A known aliquot of the borate buffer extract was wet digested using 50 % sulphuric acid followed by little dilute perchloric acid for complete oxidation, till the whole mixture turned pale green or colorless. This was made up to volume before a known amount was neutralized and its ammonia content estimated by the Berthelot reaction.

The values obtained by this procedure, denoted all soluble nitrogen components but nitrate and/or nitrite nitrogen; the latters have to be added in order to obtain the true estimate of total soluble nitrogen.

8- Inorganic Phosphorus:

The sulphite metol method, adopted by Burton and Riley (1954), was applied in this investigation. All phosphorus estimations were carried out on neutral samples using phenolphthalein as indicator.

A known volume of the neutralized algal extract was shaken with 1 ml of 2.5 % freshly prepared ammonium molybdate and 0.5 ml of 10 N sulphuric acid solutions. The mixture was kept standing for 15 minutes

before addition of 2 ml metol reagent. The mixture was diluted to the appropriate volume and its optical density was measured at 700 nm; after 30 minutes of standing.

9- Total Soluble Organic Phosphorus:

A known volume of the digest for total soluble nitrogen (item 7, P. 30) was withdrawn for the assay of its phosphorus content. If we subtract the value of inorganic phosphorus from that recorded by this procedure, we obtain the value of total soluble organic phosphorus.

B- ESTIMATION OF INSOLUBLE MATTER:

1. Total Insoluble Nitrogen:

A known volume of the sodium hydroxide - residue extract was wet digested and its nitrogen content determined following the previously mentioned procedures (item 7, p. 30).

2. Protein Nitrogen:

An aliquot of the sodium hydroxide residue extract was analysed for its protein content using the Folin phenol procedures (item 5, p. 29).

3. Other Insoluble Nitrogen:

If we subtract the value obtained by the previous

procedure from that of total insoluble nitrogen, we can easily estimate the value for other insoluble nitrogen representing the nucleoprotein nitrogen.

4- Total Insoluble Phosphorus:

A known volume of the digested sodium hydroxide - residue extract (item 1, p. 31) was neutralized before its phosphorus content was determined.

5- RNA Phosphorus:

According to Schmidt and Thanhauser (1945), 5 ml of the sodium hydroxide-residue extract were acidified with 1 ml of 6 N hydrochloric acid and 5 ml of 5 % tri-chloroacetic acid then centrifuged. Both inorganic and total phosphorus were determined in the centrifugate according to the previously mentioned procedures.

6- DNA Phosphorus:

If we subtract the total phosphorus reported by the previous procedure (item 5, p.32) from the value of the total insoluble phosphorus (item 4) we calculate the value of the DNA phosphorus in the sample.

7- Other Insoluble Phosphorus:

This is represented by the figures recorded for the

inorganic phosphorus during estimation of RNA-phosphorus (item 5, P.32).

In some instances, the phosphorus content of the samples was too low to develop its colour reaction in the previously stated periods of time. Under such conditions, the molybdate mixture was left 30 minutes before addition of the metol reagent after which the whole reaction was kept, for at least 3-4 hours, before being assayed.

P A R T I I

EFFECT OF VARIOUS RATIOS OF SULPHUR SOURCES ON
SOME GROWTH ACTIVITIES OF PHORMIDIUM
ANGUSTISSIMUM

PART II

EFFECT OF VARIOUS RATIOS OF SULPHUR SOURCES ON SOME GROWTH ACTIVITIES OF PHORMIDIUM ANGUSTISSIMUM

In this experiment we applied mixtures of sulphate ion with either thiosulphate, metabisulphite sulphite or persulphate in ratios of 80, 60, 50, 40, and 20 % of either sources. The total sulphur remained 26 mg/L as recommended by Shakeeb (1975). Control samples contained 100 % of each sulphur source. In the meantime, Phormidium was also cultured on the same medium devoid of sulphur. Under all conditions, magnesium was supplied as chloride whereas sulphur was introduced as sodium salt.

Accordingly, we had 26 variables, 15 replicates each, in order to have 5 replicates for dry weight gain, pigment estimation, nitrogen and phosphorus analysis.

1. Dry Weight Gain:

Table 1 and Fig. 1 show that (compared with sulphur free samples), sulphate, sulphite or persulphate seemed without effect whereas thiosulphate and metabisulphite stimulated such process; more prominently under the former condition. The presence of 40 % thiosulphate in

Table (1): Effect of various ratios of different sulphur sources on the biomass yield of Phormidium angustissimum (Mg d.wt. per biomass).

Treatment % SO_4^{2-} in medium	Sulphate	Thiosulphate	Meta- bisulphite	Sulphite	Per- sulphate
0	65.2±2.2	65.2±2.2	65.2±2.2	65.2±2.2	65.2±2.2
100 %	66.7±2.3	84.0±2.8	75.6±2.5	62.5±2.1	58.5±2.0
80% SO_4^{2-} + 20% other sources		81.6±2.7	75.6±2.6	81.5±2.7	66.1±2.5
60% SO_4^{2-} + 40% other sources		92.1±3.1	70.8±2.3	97.3±3.2	57.8±1.9
50% SO_4^{2-} + 50% other sources		84.3±2.8	90.2±3.0	81.5±2.7	63.3±2.2
40 % SO_4^{2-} + 60% other sources		72.0±2.4	64.8±2.1	82.5±2.8	54.4±1.8
20 % SO_4^{2-} + 80% other sources		77.6±2.6	72.0±2.4	72.4±2.5	65.6±2.4
Total sulphur = 26 mg per liter medium.					

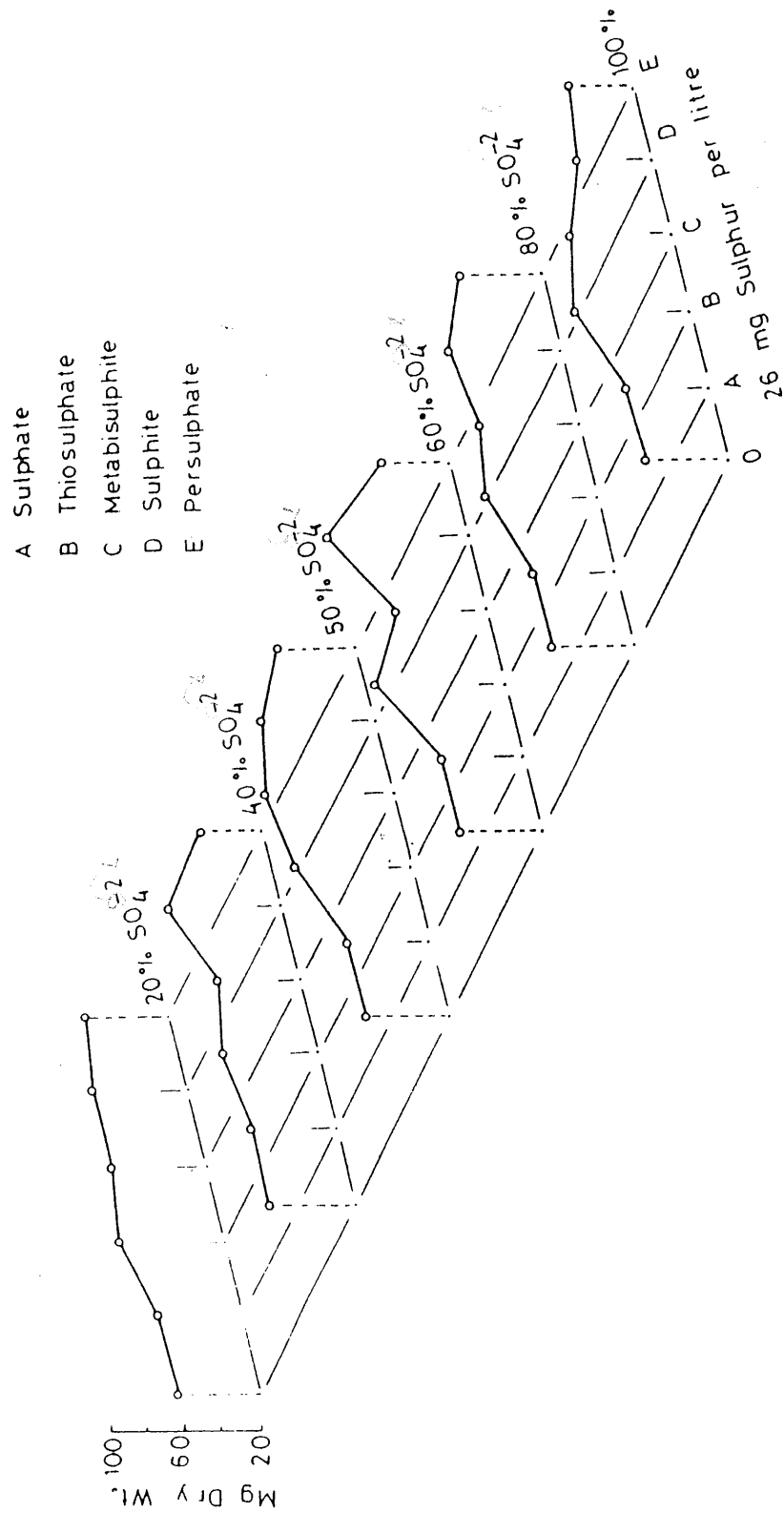


Fig.(1) Effect of various ratios of different sulphur sources on the dry weight gain by
Phormidium angustissimum.

the sulphate media seemed to stimulate dry weight gain above that of control thiosulphate; a phenomenon that declined with increasing the thiosulphate dose though still to a higher level than the control sulphate samples.

On the other hand, equal amounts of sulphate and metabisulphite favoured better gain in biomass than in presence of either ions, singly or in the other various combination, the latters seemed with little, if at all, effect compared with either controls. Mixing sulphate with sulphite initiated better dry weight yield than supplementation of each sulphur source alone; a phenomenon that was most apparent at 40 % sulphite that induced the largest biomass among all mixtures. On the contrary, coupling sulphate with persulphate seemed with minor effect on dry weight gain compared with supplementation of either sources alone.

2. Pigmentation:

Table 2 and Fig. 2 show that sulphite or persulphate equally stimulated whereas sulphate and thiosulphate were inhibitory (the latter being more effective) to chlorophyll a accumulation, but metabisulphite seemed without

Table (2): Effect of various ratios of different sulphur sources on chlorophyll a content of Phormidium angustissimum (Mg per 1 g D. wt.)

Treatment % SO ₄ in medium	Sulphate	Thio- sulphate	Meta- bisulphite	Sulphite	Per- sulphate
0	3.56±0.12	3.56±0.12	3.56±0.12	3.56±0.12	3.56±0.12
100 %	2.86±0.10	1.41±0.05	3.30±0.12	4.10±0.14	4.04±0.13
80% SO ₄ + 20% other sources		2.37±0.09	2.75±0.10	3.14±0.11	4.61±0.16
60% SO ₄ + 40% other sources		1.37±0.05	2.80±0.12	2.71±0.10	5.56±0.18
50% SO ₄ + 50% other sources		1.39±0.06	2.36±0.10	2.19±0.09	4.44±0.15
40% SO ₄ + 60% other sources		1.50±0.06	3.05±0.10	2.17±0.10	4.74±0.16
20% SO ₄ + 80% other sources		1.24±0.05	3.39±0.12	3.55±0.13	3.45±0.12

Total sulphur = 26 mg per liter medium.

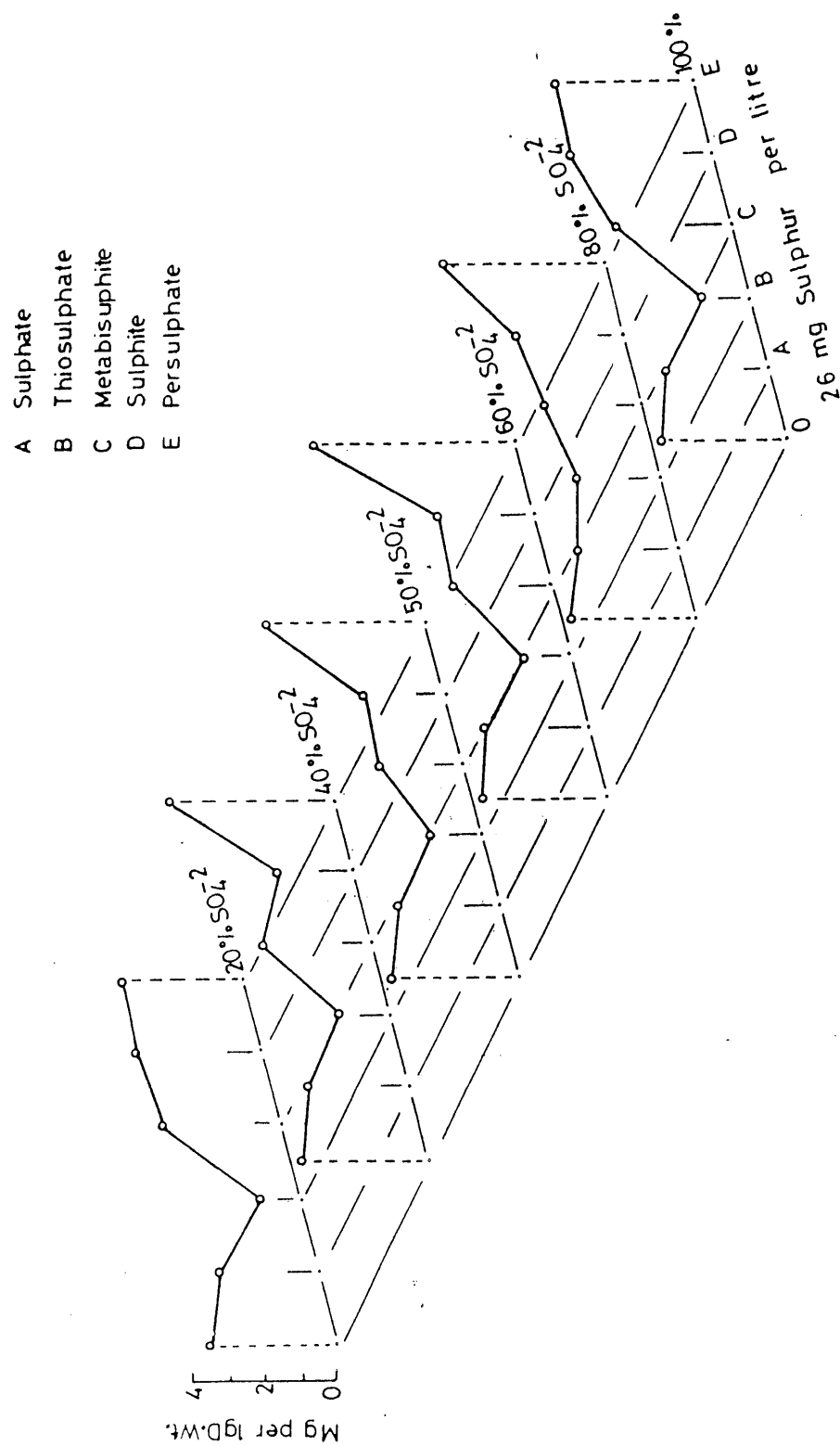


Fig. (2) Effect of various ratios on different sulphur sources on chlorophyll a content of Phormidium angustissimum.

effect compared to control samples free of sulphur source. Addition of thiosulphate to sulphate media attenuated chlorophyll a formation; with minor differences between various treatments above 20 % level of thiousulphate.

On the other hand, the smaller percentage of either sulphate or metabisulphite, in their mixture; seemed without effect whereas equal amounts of both ions inhibited chlorophyll a accumulation in the algal biomass. In the meantime, equal levels of sulphate and sulphite ions favoured a steep drop in chlorophyll a content that was partially alleviated when larger percentages of either ions was prevailing. Coupling sulphate with persulphate initiated the largest accumulation of chlorophyll a especially at 40 % of either ions in the media.

Table 3 and Fig. 3 show that sulphite retarded whereas metabisulphite and persulphate stimulated (the former being less effective) carotenoid formation but sulphate or thiosulphate seemed without effect, compared with the algal samples deprived of sulphur. Coupling thiosulphate with sulphate highly inhibited carotenoid accumulation; more prominently with larger doses of the former, whereas addition of metabisulphite to sulphate media was not

Table (3): Effect of various ratios of different sulphur sources on carotenoid content of Phormidium angustissimum (Mg per 1 g D. wt.).

Treatment % SO ₄ ²⁻ in medium	Sulphate	Thio sulphate	Meta- bisulphite	Sulphite	Per- sulphate
0	0.26±0.03	0.26±0.03	0.26±0.03	0.26±0.03	0.26±0.03
100 %	0.29±0.03	0.22±0.02	0.44±0.04	0.17±0.02	0.80±0.05
80% SO ₄ ²⁻ + 20% other sources		0.66±0.05	0.29±0.03	0.30±0.03	1.15±0.06
60% SO ₄ ²⁻ + 40% other sources		1.50±0.07	0.38±0.03	0.50±0.04	1.04±0.05
50% SO ₄ ²⁻ + 50% other sources		1.22±0.05	0.34±0.03	0.42±0.04	1.48±0.06
40% SO ₄ ²⁻ + 60% other sources		1.43±0.06	0.26±0.03	0.33±0.03	1.03±0.05
20% SO ₄ ²⁻ + 80% other sources		1.35±0.05	0.29±0.03	0.12±0.02	0.90±0.05

Total sulphur = 26 mg per liter medium.

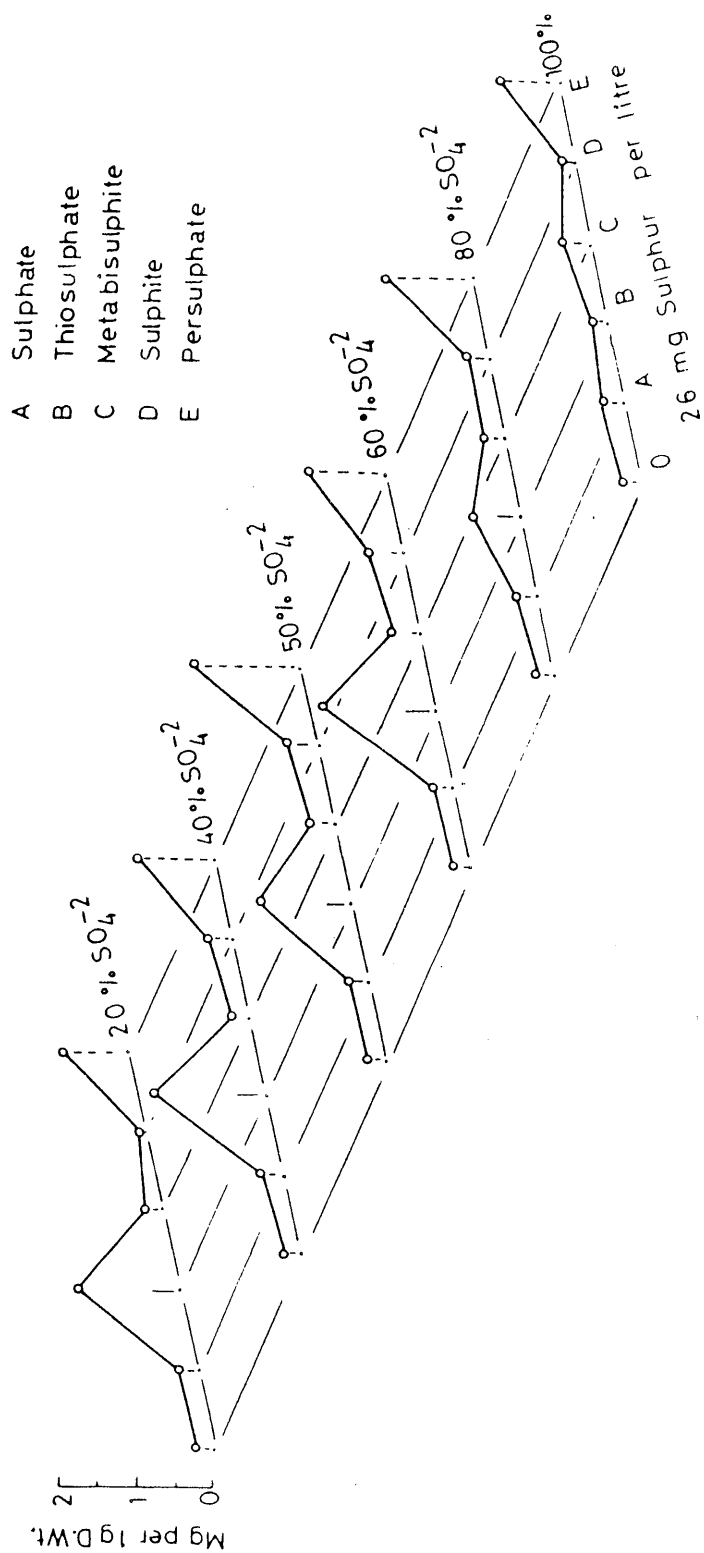


Fig.(3) Effect of various ratios of different sulphur sources on the Carotenoids content of Phormidium angustissimum

effective. Equal amounts of sulphate and sulphite favoured better carotenoid accumulation but larger percentage of either sulphur sources restored the carotenoid level to that of the respective ion. In the meantime, equal doses of sulphate and persulphate favoured the highest carotenoid accumulation; a phenomenon that was partially counteracted with rise of concentration of either ions.

3- Nitrate Disappearance:

Table 4 shows that nitrate disappearance was invariably affected by the presence or absence of sulphur in the media except for thiosulphate that reduced the amount of absorbed nitrogen by the alga. 80 % either sulphate or thiosulphate did not alter nitrogen uptake compared with pure sulphate media whereas 60 % thiosulphate remarkably enhanced such process. The remaining combinations favoured a low absorption rate similar to that reported for pure thiosulphate media. Coupling sulphate with metabisulphite hardly affected disappearance except when equal ratio of both sources was prevailing where a noticeable drop was recorded. Similarly, equal proportions of sulphate and sulphite induced a severe repression of nitrate uptake; a phenomenon that was partially alleviated with

Table (4): Effect of various ratios of different sulphur sources on nitrate disappearance from the media of Phormidium angustissimum (mgm per 1 gm dry weight).

Treatment % SO ₄ ²⁻ in medium	Sulphate	Thio- sulphate	Meta- bisulphite	Sulphite	Persulphate
0	78.12±2.57	78.12±2.57	78.12±2.57	78.12±2.57	78.12±2.57
100 %	71.96±2.38	61.43±2.02	72.01±2.39	78.97±2.61	73.14±2.41
80% SO ₄ ²⁻ + 20 % other sources		71.55±2.36	66.86±2.22	58.39±1.93	75.68±2.50
60% SO ₄ ²⁻ + 40 % other sources		66.17±2.19	65.45±2.16	52.32±1.73	85.24±2.82
50% SO ₄ ²⁻ + 50 % other sources		60.03±1.98	59.39±1.96	49.27±1.63	88.89±2.94
40% SO ₄ ²⁻ + 60 % other sources		85.62±2.84	66.06±2.18	61.28±2.03	82.97±2.74
20% SO ₄ ²⁻ + 80 % other sources		72.43±2.39	69.29±2.87	70.51±2.33	74.95±2.48

Total sulphur = 26 mg per litre medium.

increasing the dose of either ions, particularly sulphite. On the other hand, the range of 40 - 60% of either sulphate or persulphate, in their mixtures, increased disappearance whereas the larger percentages seemed without effect compared with 100 % of either ions.

4- Nitrogen Secretion:

Table 5 and Fig. 4 show that addition of sulphur to the nutrient media lowered nitrogen secretion; more prominently when thiosulphate was administered followed by sulphate and least by either of the remaining sources. 40-50 % thiosulphate in sulphate media restored nitrogen secretion to that of pure thiosulphate media whereas the other ratios had no effect compared to pure sulphate. Coupling metabisulphite, sulphite or persulphate with sulphate initiated better nitrogen secretion compared with sulphate alone; a phenomenon that was furthered with increased ratio of metabisulphite or persulphate; more prominently the latter source but slightly attenuated with larger proportion of sulphite ion.

It is interesting to note that all thiosulphate media were free of ammonia, whereas all sulphite and

Table (5): Effect of various ratios of different sulphur sources on nitrogen secretion by Phormidium aurustissimum (mg per 1 gm dry weight)

% Sulphur	Sulphur source	Nitrite N	Ammonia N	Amide N	Amino N	Peptide N	Other Soluble N	Total N
0	0	1.81 ± 0.07	0.13 ± 0.01	0.18 ± 0.02	1.65 ± 0.07	4.85 ± 0.16	9.19 ± 0.31	17.81 ± 0.59
100 %	Sulphate	1.48 ± 0.06	0.12 ± 0.01	0.14 ± 0.01	1.82 ± 0.07	3.80 ± 0.13	5.61 ± 0.18	12.97 ± 0.43
	Thiosulphate	0.79 ± 0.04	-	0.20 ± 0.02	0.29 ± 0.02	3.02 ± 0.11	4.89 ± 0.16	9.19 ± 0.30
	Metabisulphite	0.95 ± 0.04	0.08 ± 0.01	0.06 ± 0.01	-	5.49 ± 0.18	8.48 ± 0.28	15.06 ± 0.50
	Sulphite	2.67 ± 0.10	0.03 ± 0.01	-	0.96 ± 0.04	5.34 ± 0.17	6.16 ± 0.21	15.16 ± 0.51
-	Persulphate	1.62 ± 0.06	0.24 ± 0.02	-	1.89 ± 0.08	4.18 ± 0.14	7.75 ± 0.26	15.68 ± 0.51
80 % Sulphate + 20 % of	Thiosulphate	1.18 ± 0.05	-	0.16 ± 0.01	1.40 ± 0.06	2.77 ± 0.10	5.89 ± 0.19	11.40 ± 0.37
	Metabisulphite	1.02 ± 0.04	0.22 ± 0.02	0.10 ± 0.01	1.24 ± 0.05	3.59 ± 0.12	5.19 ± 0.30	15.36 ± 0.51
	Sulphite	1.80 ± 0.06	0.11 ± 0.02	-	1.62 ± 0.06	3.26 ± 0.11	6.66 ± 0.23	13.45 ± 0.44
	Persulphate	2.03 ± 0.08	0.18 ± 0.02	-	1.78 ± 0.07	4.96 ± 0.17	9.49 ± 0.31	18.44 ± 0.61
60 % Sulphate + 40 % of	Thiosulphate	1.02 ± 0.04	-	0.48 ± 0.03	1.32 ± 0.05	2.14 ± 0.09	4.36 ± 0.15	9.32 ± 0.31
	Metabisulphite	1.02 ± 0.03	0.18 ± 0.02	0.11 ± 0.01	1.05 ± 0.04	3.81 ± 0.13	11.20 ± 0.37	17.37 ± 0.57
	Sulphite	1.52 ± 0.06	0.09 ± 0.02	-	1.38 ± 0.05	3.09 ± 0.10	6.80 ± 0.22	12.88 ± 0.42
	Persulphate	1.56 ± 0.05	0.21 ± 0.02	-	1.68 ± 0.07	5.32 ± 0.18	11.09 ± 0.37	19.86 ± 0.66
50 % Sulphate + 50 % of	Thiosulphate	0.87 ± 0.04	-	0.24 ± 0.02	1.38 ± 0.04	2.68 ± 0.10	3.73 ± 0.12	8.90 ± 0.30
	Metabisulphite	0.92 ± 0.04	0.19 ± 0.02	0.09 ± 0.01	0.83 ± 0.03	4.18 ± 0.14	10.01 ± 0.33	16.22 ± 0.54
	Sulphite	1.54 ± 0.06	0.11 ± 0.02	-	0.73 ± 0.03	3.68 ± 0.13	6.55 ± 0.22	12.61 ± 0.42
	Persulphate	1.81 ± 0.07	0.15 ± 0.02	-	1.61 ± 0.06	4.20 ± 0.14	14.39 ± 0.48	22.16 ± 0.73
40 % Sulphate + 60 % of	Thiosulphate	0.93 ± 0.04	-	0.36 ± 0.03	0.90 ± 0.04	3.78 ± 0.13	5.20 ± 0.18	11.19 ± 0.37
	Metabisulphite	1.13 ± 0.05	0.08 ± 0.02	0.07 ± 0.02	0.62 ± 0.03	5.75 ± 0.19	9.30 ± 0.31	16.95 ± 0.56
	Sulphite	1.59 ± 0.05	0.11 ± 0.02	-	0.73 ± 0.03	4.45 ± 0.15	7.24 ± 0.24	14.12 ± 0.46
	Persulphate	1.70 ± 0.06	0.16 ± 0.02	-	1.69 ± 0.06	4.50 ± 0.16	16.95 ± 0.56	25.00 ± 0.83
20 % Sulphate + 80 % of	Thiosulphate	1.02 ± 0.04	-	0.44 ± 0.03	0.86 ± 0.04	2.90 ± 0.10	6.97 ± 0.23	12.19 ± 0.40
	Metabisulphite	1.14 ± 0.04	0.07 ± 0.02	0.09 ± 0.02	-	5.19 ± 0.17	9.99 ± 0.33	16.46 ± 0.54
	Sulphite	2.11 ± 0.08	0.06 ± 0.02	-	0.83 ± 0.04	4.63 ± 0.15	6.81 ± 0.23	14.46 ± 0.48
	Persulphate	2.19 ± 0.08	0.16 ± 0.02	-	1.73 ± 0.07	4.38 ± 0.14	16.06 ± 0.53	24.52 ± 0.81

Total sulphur = 26 mg per liter medium.

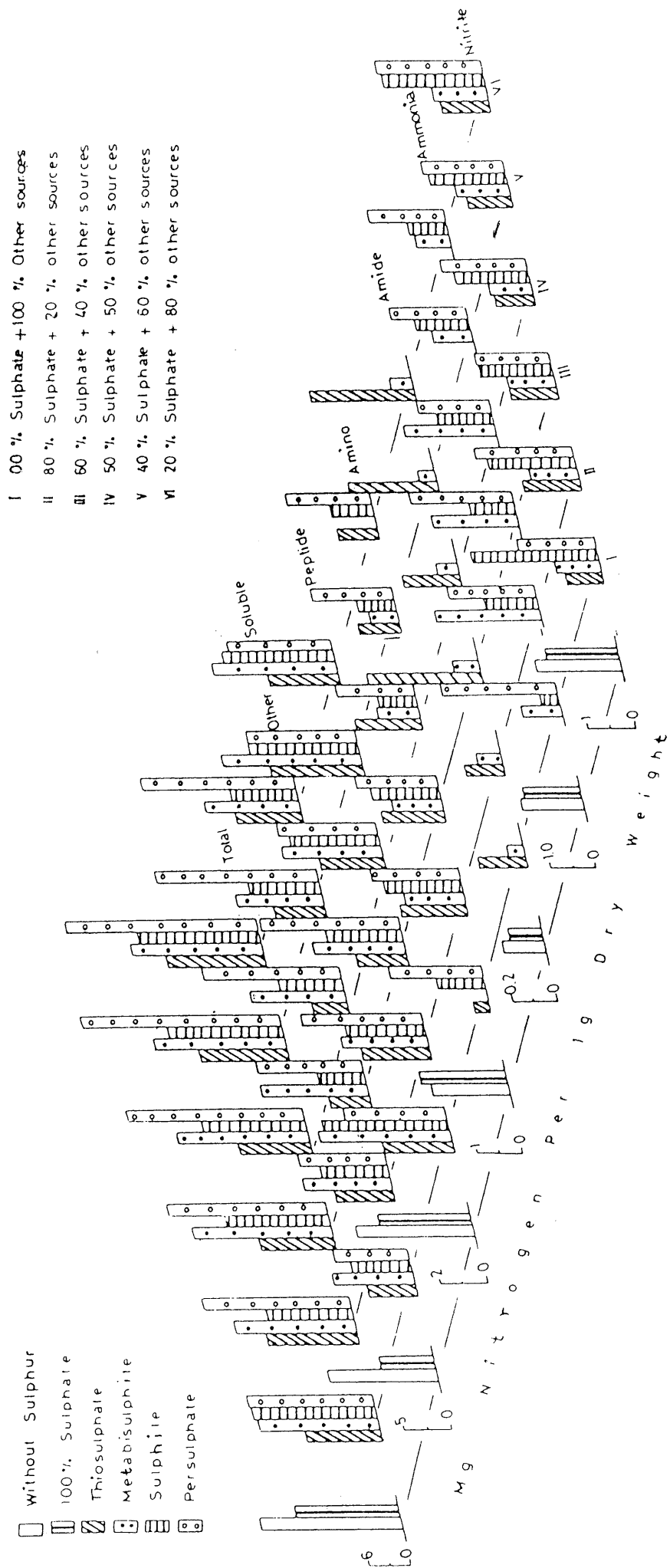


Fig.(4) Effect of various ratios of different sulphur sources on nitrogen secretion by *Phormidium angustissimum*

persulphate media were devoid of amide nitrogen. In the meantime, although all components shared in the forementioned fluctuations in total nitrogen secretion, yet the change in peptide and/or other soluble nitrogen were the most prominent.

Irrespective of such fluctuations the percentage nitrogen secretion (Table 6) was almost unaffected by the various sulphur treatments except with thiosulphate, in the media, where a noticeable drop was noticed as well as with large proportions of persulphate in sulphate media where a remarkable increase was observed. 100 % sulphate also favoured lower ratio of nitrogen secretion compared to sulphur free media.

5- Nitrogen Components of Biomass:

Table 7 and Fig. 5 show that the presence of either sulphur sources in the media hardly affected the total nitrogen content of the biomass except for a slight depression, with administration of thiosulphate; as a result of low soluble components and protein accompanied by increased other soluble nitrogen. Metabisulphite ion increased nitrate and other insoluble nitrogen on the

(3)

Table (6): Effect of various ratios of different sulphur sources on percentage nitrogen secretion by Phormidium angustissimum

Treatment % SO ₄ ²⁻ in medium	Sulphate	Thio sulphate	Meta- bisulphite	Sulphite	Persulphate
0	22.80	22.80	22.80	22.80	22.80
100 %	18.02	14.96	20.91	19.20	21.44
80% SO ₄ ²⁻ + 20% other sources		15.93	22.97	23.03	24.37
60% SO ₄ ²⁻ + 40% other sources		14.08	26.54	24.62	23.30
50% SO ₄ ²⁻ + 50% other sources		14.82	27.31	25.59	24.93
40% SO ₄ ²⁻ + 60% other sources		13.07	25.66	23.04	30.13
20% SO ₄ ²⁻ + 80% other sources		16.83	23.78	20.51	32.72

Total sulphur = 26 mg per litre medium.

Table (7): Effect of various ratios of different sulphur sources on the nitrogen components of *Phormidium angustissimum* (mg per 1 gm dry weight).

% Sulphur	Sulphur source	Nitrate N	Ammonia N	Amino N	Peptide N	Other Soluble N	Total Soluble N	Protein N	Other Insoluble N	Total Insoluble N	Total N
0	0	2.25 ± 0.08	0.34 ± 0.03	3.84 ± 0.27	7.12 ± 0.25	8.51 ± 0.30	22.06 ± 0.74	18.68 ± 0.63	18.84 ± 0.64	37.52 ± 1.24	59.48 ± 1.92
100 %	Sulphate	2.13 ± 0.08	0.24 ± 0.02	2.35 ± 0.09	6.03 ± 0.21	11.83 ± 0.40	22.58 ± 0.76	17.87 ± 0.61	17.62 ± 0.59	35.49 ± 1.17	58.07 ± 1.90
	Thiosulphate	1.12 ± 0.05	0.16 ± 0.02	2.28 ± 0.09	2.01 ± 0.08	14.02 ± 0.47	19.55 ± 0.66	14.52 ± 0.50	17.33 ± 0.58	31.85 ± 1.10	51.44 ± 1.69
	Metabisulphite	3.09 ± 0.11	0.26 ± 0.02	2.13 ± 0.08	6.73 ± 0.23	7.01 ± 0.24	19.22 ± 0.64	12.89 ± 0.45	23.03 ± 0.77	35.91 ± 1.19	55.31 ± 1.80
	Sulphite	2.57 ± 0.09	0.38 ± 0.03	3.19 ± 0.12	19.41 ± 0.65	5.08 ± 0.18	30.63 ± 1.02	22.75 ± 0.76	10.98 ± 0.38	33.73 ± 1.11	64.36 ± 2.11
	Persulphate	2.33 ± 0.09	0.24 ± 0.02	1.76 ± 0.07	1.77 ± 0.07	7.85 ± 0.27	13.95 ± 0.47	18.56 ± 0.64	23.63 ± 0.79	42.19 ± 1.39	56.14 ± 0.84
80 % Sulphate + 20 % of	Thiosulphate	0.86 ± 0.04	0.33 ± 0.02	3.60 ± 0.13	2.41 ± 0.04	21.84 ± 0.73	29.04 ± 0.97	13.56 ± 0.46	15.93 ± 0.51	29.49 ± 0.97	58.53 ± 1.91
	Metabisulphite	2.99 ± 0.11	0.20 ± 0.02	1.71 ± 0.07	5.63 ± 0.20	6.07 ± 0.21	16.60 ± 0.58	14.58 ± 0.50	20.88 ± 0.70	35.44 ± 1.16	52.04 ± 1.81
	Sulphite	1.23 ± 0.05	0.33 ± 0.03	2.25 ± 0.08	12.78 ± 0.43	5.96 ± 0.21	22.55 ± 0.76	15.74 ± 0.53	5.76 ± 0.22	21.50 ± 0.72	44.05 ± 1.45
	Persulphate	4.47 ± 0.16	0.17 ± 0.02	2.13 ± 0.08	2.08 ± 0.08	5.98 ± 0.21	14.83 ± 0.51	19.99 ± 0.67	21.25 ± 0.72	41.24 ± 1.35	56.07 ± 1.84
60 % Sulphate + 40 % of	Thiosulphate	0.81 ± 0.04	0.18 ± 0.02	3.86 ± 0.13	1.89 ± 0.07	21.87 ± 0.63	28.61 ± 0.96	13.19 ± 0.46	15.71 ± 0.53	28.90 ± 0.95	57.51 ± 1.88
	Metabisulphite	3.18 ± 0.11	0.24 ± 0.02	1.70 ± 0.07	5.42 ± 0.19	6.52 ± 0.23	17.06 ± 0.57	15.62 ± 0.53	13.94 ± 0.47	29.54 ± 0.97	46.60 ± 1.51
	Sulphite	1.40 ± 0.06	0.36 ± 0.03	1.65 ± 0.07	11.42 ± 0.39	3.34 ± 0.12	18.17 ± 0.61	13.53 ± 0.47	6.54 ± 0.24	20.07 ± 0.66	38.24 ± 1.25
	Persulphate	4.17 ± 0.15	0.18 ± 0.02	2.69 ± 0.10	2.38 ± 0.09	6.23 ± 0.22	16.65 ± 0.56	19.29 ± 0.66	28.63 ± 0.96	47.98 ± 1.58	64.63 ± 2.11
50 % Sulphate + 50 % of	Thiosulphate	0.85 ± 0.04	0.23 ± 0.02	2.19 ± 0.08	2.08 ± 0.08	15.89 ± 0.53	21.24 ± 0.71	12.83 ± 0.43	15.82 ± 0.54	28.65 ± 0.95	49.89 ± 1.61
	Metabisulphite	2.26 ± 0.08	0.21 ± 0.02	1.41 ± 0.06	4.50 ± 0.16	5.64 ± 0.20	14.02 ± 0.49	11.61 ± 0.39	15.36 ± 0.55	27.57 ± 0.91	41.59 ± 1.35
	Sulphite	1.26 ± 0.06	0.35 ± 0.03	2.01 ± 0.08	11.72 ± 0.40	2.31 ± 0.10	17.65 ± 0.58	12.52 ± 0.44	5.36 ± 0.21	17.88 ± 0.59	35.53 ± 1.14
	Persulphate	4.03 ± 0.14	0.17 ± 0.02	2.24 ± 0.09	2.33 ± 0.09	7.81 ± 0.26	16.58 ± 0.55	18.26 ± 0.62	32.29 ± 1.10	50.65 ± 1.67	67.23 ± 2.19
40 % Sulphate + 60 % of	Thiosulphate	0.91 ± 0.04	0.26 ± 0.02	2.89 ± 0.11	2.36 ± 0.10	23.49 ± 0.78	29.91 ± 0.99	14.97 ± 0.52	27.75 ± 0.95	42.72 ± 1.41	72.63 ± 2.38
	Metabisulphite	2.99 ± 0.11	0.22 ± 0.02	1.83 ± 0.07	7.06 ± 0.24	6.58 ± 0.24	18.68 ± 0.62	14.48 ± 0.50	14.39 ± 0.50	28.87 ± 0.95	47.55 ± 1.59
	Sulphite	1.59 ± 0.07	0.19 ± 0.02	2.33 ± 0.09	13.94 ± 0.48	3.51 ± 0.14	21.56 ± 0.71	17.30 ± 0.59	7.13 ± 0.25	24.43 ± 0.81	45.99 ± 1.50
	Persulphate	3.22 ± 0.12	0.19 ± 0.02	2.27 ± 0.09	2.08 ± 0.08	6.81 ± 0.23	14.57 ± 0.48	17.78 ± 0.61	27.13 ± 0.92	44.93 ± 1.48	59.50 ± 1.95
20 % Sulphate + 80 % of	Thiosulphate	1.16 ± 0.05	0.26 ± 0.02	5.00 ± 0.18	2.25 ± 0.09	19.39 ± 0.66	28.06 ± 0.92	12.79 ± 0.44	18.19 ± 0.63	30.98 ± 1.02	59.04 ± 1.90
	Metabisulphite	3.39 ± 0.13	0.21 ± 0.02	2.08 ± 0.08	6.94 ± 0.24	7.69 ± 0.26	20.31 ± 0.67	12.58 ± 0.44	18.58 ± 0.64	31.16 ± 1.03	51.47 ± 1.73
	Sulphite	2.19 ± 0.08	0.28 ± 0.03	2.65 ± 0.10	16.87 ± 0.57	3.56 ± 0.14	25.55 ± 0.84	19.49 ± 0.66	9.39 ± 0.33	28.88 ± 0.95	54.47 ± 1.82
	Persulphate	2.76 ± 0.10	0.23 ± 0.02	2.07 ± 0.08	1.93 ± 0.07	7.12 ± 0.25	14.11 ± 0.47	17.58 ± 0.60	25.41 ± 0.87	42.99 ± 1.42	57.10 ± 1.85

Total Sulphur = 26 mg per liter medium.

expense of other soluble and protein nitrogen. On the other hand, sulphite ion enhanced peptide and protein accumulation with a concomitant drop in other insoluble nitrogen. In the meantime, persulphate severely attenuated amino and peptide nitrogen with a remarkable rise in the other soluble component.

The presence of thiosulphate ion in sulphate media did not affect the total nitrogen content of the biomass, compared to 100 % sulphate-fed samples except at equal proportions of both ions or 60 % thiosulphate where a drop to 100 % thiosulphate-fed samples, under the former, and a remarkable increase, under the latter condition; was noticed. Within all mixtures, the nitrate, peptide and protein content did not exceed that under pure thiosulphate feeding whereas amino, other soluble and other insoluble nitrogen largely increased.

The presence of metabisulphite in sulphate media favoured a low total nitrogen content particularly at 40 % level or more; the drop being mainly in amino, peptide, other soluble and/or protein nitrogen coupled with increased ammonia level. Equal proportions of sulphite

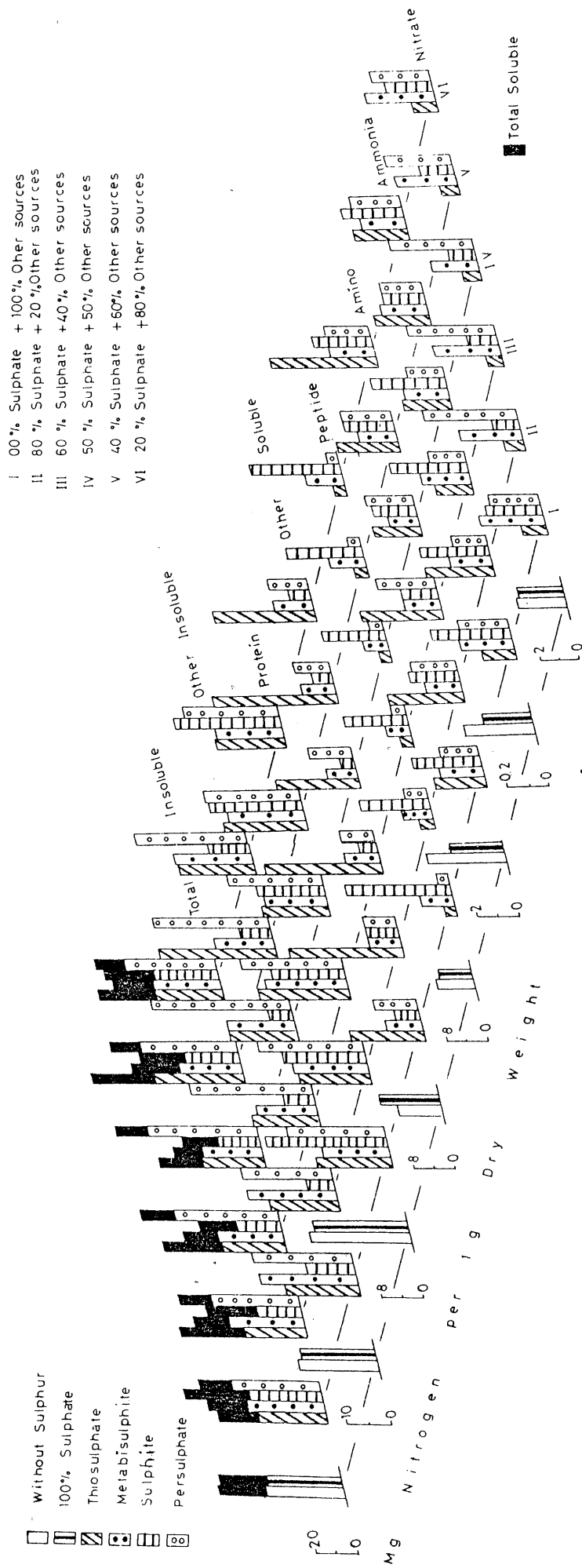


Fig. (5) Effect of various ratios of different sulphur sources on the nitrogen components of biomass of *Phormidium angustissimum*.

and sulphate induced a severe drop in total nitrogen content; a phenomenon that was almost alleviated with increased percentage of either sulphur sources particularly sulphite. Under all conditions, a noticeable drop in all nitrogen components was observed except for peptide and protein nitrogen that largely increased. The presence of persulphate in the sulphate media, though had little, if at all, effect on total nitrogen content yet it still lowered the peptide content with a remarkable increase in ammonia and other insoluble nitrogen.

6- Phosphorus Components of Biomass:

Table 8 and Fig. 6 show that neither sulphate nor sulphite ions affected the total phosphorus content of the algal biomass whereas the other sulphur sources were suppressive; especially thiosulphate; compared with samples deficient in sulphur. Under all conditions, the drop was mainly in the insoluble components and/or soluble organic phosphorus.

Addition of larger proportions of thiosulphate to the sulphate media favoured less total phosphorus content, compared with pure sulphate media; a phenomenon that was

Table (8): Effect of various ratios of different sulphur sources on the phosphorus components of *Phormidium angustissimum* (mg per 1 gm dry weight)

% Sulphur	Sulphur Source	Inorganic P	Soluble Organic P	Total Sol P	RNA P	DNA P	Other Insol. P	Total Insol. P	Total P
0	0	8.91 ± 0.31	19.11 ± 0.65	28.02 ± 0.93	3.06 ± 0.14	7.55 ± 0.27	2.54 ± 0.11	13.15 ± 0.46	41.17 ± 1.33
100 %	Sulphate	9.56 ± 0.35	17.22 ± 0.59	26.78 ± 0.89	2.59 ± 0.10	7.94 ± 0.28	1.96 ± 0.09	12.49 ± 0.44	39.27 ± 1.25
	Thiosulphate	4.42 ± 0.17	12.16 ± 0.41	16.58 ± 0.55	1.33 ± 0.06	4.75 ± 0.19	1.03 ± 0.05	7.11 ± 0.25	23.69 ± 0.72
	Metabisulphite	8.12 ± 0.29	13.21 ± 0.45	21.33 ± 0.70	1.00 ± 0.05	5.47 ± 0.21	1.55 ± 0.07	8.02 ± 0.27	29.35 ± 0.92
	Sulphite	12.33 ± 0.43	14.61 ± 0.50	26.94 ± 0.89	3.75 ± 0.15	13.33 ± 0.46	2.27 ± 0.10	19.35 ± 0.66	46.29 ± 1.47
80 %	Persulphate	7.46 ± 0.27	20.55 ± 0.67	28.01 ± 0.91	0.77 ± 0.05	3.08 ± 0.12	1.41 ± 0.07	5.26 ± 0.21	33.27 ± 1.05
	Thiosulphate	4.24 ± 0.17	20.40 ± 0.76	24.64 ± 0.84	1.41 ± 0.07	8.74 ± 0.32	1.37 ± 0.06	11.52 ± 0.39	36.16 ± 1.15
	Metabisulphite	8.00 ± 0.29	14.53 ± 0.51	22.63 ± 0.76	1.66 ± 0.08	6.27 ± 0.24	1.42 ± 0.07	9.35 ± 0.33	31.98 ± 1.05
	Sulphite	10.66 ± 0.37	17.35 ± 0.60	28.01 ± 0.88	3.23 ± 0.14	14.50 ± 0.50	1.81 ± 0.09	19.54 ± 0.68	45.86 ± 1.45
60 %	Persulphate	4.02 ± 0.16	21.67 ± 0.75	32.33 ± 1.08	1.33 ± 0.07	6.84 ± 0.25	2.63 ± 0.12	10.60 ± 0.37	42.93 ± 1.40
	Thiosulphate	7.43 ± 0.27	18.80 ± 0.66	22.82 ± 0.77	1.25 ± 0.06	8.95 ± 0.31	1.34 ± 0.05	11.54 ± 0.40	34.36 ± 1.12
	Metabisulphite	4.47 ± 0.35	13.61 ± 0.47	21.04 ± 0.69	1.31 ± 0.06	5.80 ± 0.22	1.36 ± 0.06	8.47 ± 0.30	29.51 ± 0.95
	Sulphite	14.36 ± 0.50	21.54 ± 0.74	35.90 ± 1.20	2.76 ± 0.12	13.05 ± 0.45	0.99 ± 0.05	16.80 ± 0.57	37.97 ± 1.25
50 %	Persulphate	4.27 ± 0.18	16.45 ± 0.57	20.72 ± 0.71	1.61 ± 0.08	11.14 ± 0.39	1.32 ± 0.05	14.07 ± 0.48	34.79 ± 1.13
	Thiosulphate	8.51 ± 0.31	12.50 ± 0.45	21.01 ± 0.73	0.91 ± 0.05	5.32 ± 0.20	1.76 ± 0.08	7.99 ± 0.28	29.00 ± 0.96
	Metabisulphite	9.39 ± 0.34	12.05 ± 0.44	21.44 ± 0.72	2.12 ± 0.09	12.32 ± 0.44	1.25 ± 0.06	15.69 ± 0.54	37.13 ± 1.22
	Sulphite	13.11 ± 0.46	22.55 ± 0.76	35.66 ± 1.18	2.84 ± 0.11	5.99 ± 0.22	1.69 ± 0.07	10.52 ± 0.37	46.18 ± 1.50
40 %	Persulphate	3.95 ± 0.15	15.84 ± 0.56	19.79 ± 0.68	1.42 ± 0.06	8.54 ± 0.31	1.12 ± 0.05	11.08 ± 0.38	30.87 ± 1.12
	Thiosulphate	10.46 ± 0.37	13.21 ± 0.46	23.67 ± 0.79	1.05 ± 0.05	5.76 ± 0.21	2.09 ± 0.09	8.90 ± 0.33	32.57 ± 1.08
	Metabisulphite	9.49 ± 0.33	12.73 ± 0.45	22.22 ± 0.75	2.70 ± 0.10	11.67 ± 0.42	1.43 ± 0.06	15.80 ± 0.53	38.02 ± 1.24
	Sulphite	14.61 ± 0.51	28.12 ± 0.95	42.83 ± 1.44	1.69 ± 0.08	5.20 ± 0.21	2.02 ± 0.10	8.91 ± 0.32	51.74 ± 1.70
20 %	Persulphate	3.99 ± 0.14	15.03 ± 0.52	19.02 ± 0.65	1.19 ± 0.05	6.15 ± 0.25	1.12 ± 0.05	8.46 ± 0.31	27.48 ± 0.89
	Thiosulphate	9.91 ± 0.35	12.88 ± 0.44	22.79 ± 0.76	1.00 ± 0.04	6.06 ± 0.24	1.73 ± 0.08	8.79 ± 0.32	31.58 ± 1.04
	Metabisulphite	9.51 ± 0.34	14.27 ± 0.48	23.78 ± 0.80	3.15 ± 0.12	12.58 ± 0.44	1.61 ± 0.08	17.34 ± 0.59	41.12 ± 1.35
	Sulphite	10.64 ± 0.37	24.84 ± 0.85	35.48 ± 1.17	0.93 ± 0.04	3.91 ± 0.16	1.40 ± 0.06	6.24 ± 0.25	41.72 ± 1.37

Total sulphur = 26 mg per liter medium.

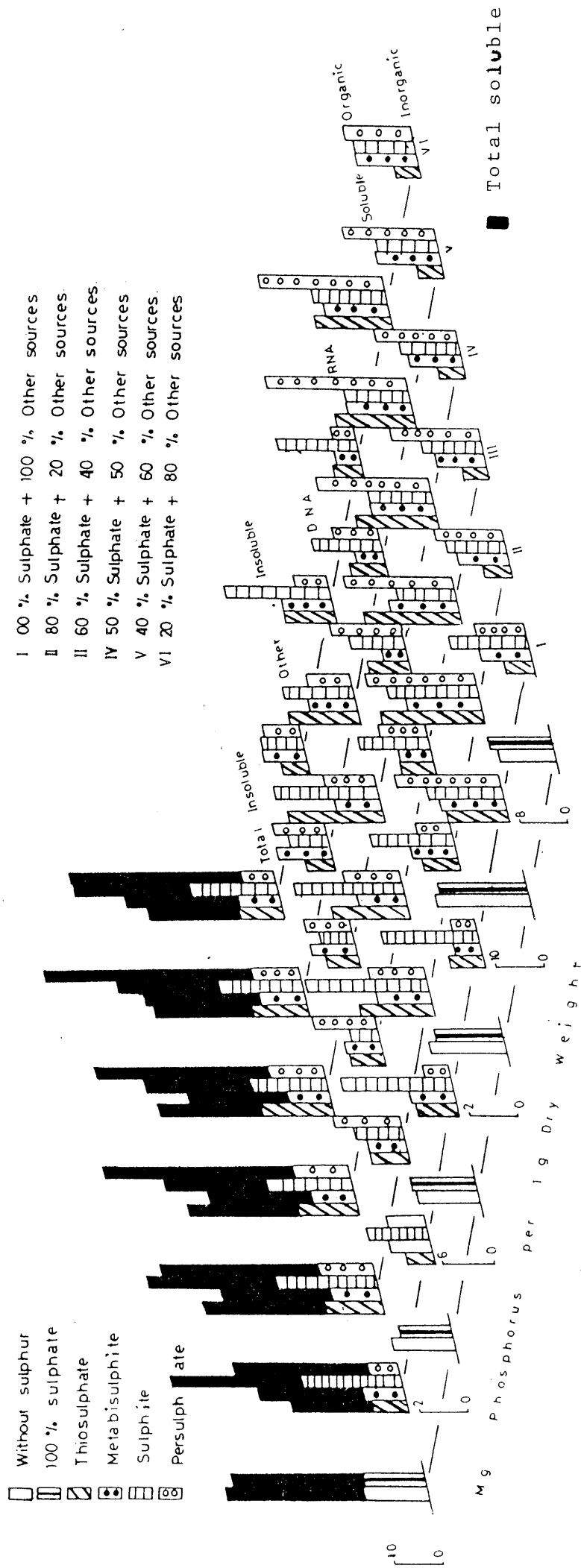


Fig.(6) Effect of various ratio of different sulphur sources on the phosphorus components of *Phormidium angustissimum*

gradually alleviated with rise of sulphate concentration. The shortage was mainly in inorganic phosphorus, RNA and other insoluble. On the other hand, the drop in total phosphorus, following metabisulphite addition to sulphate media, was consistent irrespective of variation of ratio of either sulphur sources. Fluctuations in RNA, other insoluble phosphorus and/or soluble organic phosphorus were responsible for such effect.

In the meantime, though coupling sulphite with sulphate hardly affected the total phosphorus content, yet DNA content remarkably increased on the expense of reduction, mainly in other insoluble phosphorus followed, in few cases, by organic phosphorus. Similarly, persulphate had little, if at all, effect on total phosphorus, when coupled with sulphate but the soluble components remarkably increased on the expense of the insoluble fraction.

PART III

DISCUSSION

PART III

DISCUSSION

The results of this investigation clearly establish the fact that growth of Phormidium angustissimum was not affected by absence of sulphur as a nutrient element. Thio-sulphate seemed to be a better source of sulphur for maintaining maximum growth vigour of the organism by initiating maximum gain in dry weight, followed by metabisulphite whereas sulphite, sulphate or persulphate hardly affected growth though the latter source tended to be inhibitory compared to sulphur deficient samples.

These observations clearly show that dithionates are more effective sulphur source than monothionates, for Phormidium; more prominently at their lower than higher states of oxidation i.e. with increasing their redox potential or number of lone pairs. This indicates the involvement of such ions in the pathways of sulphur metabolism by this organism.

These observations differ, in one way or the other, from those recorded for higher or other lower plants. It is well established that sulphur dioxide or sulphite ion are quite injurious to a variety of higher plants

(Miyake and Uno, 1973; Markowski et al., 1975; Ferguson et al., 1978; Ashenden, 1978; 1979; Kord, 1981). Among fungi; Mehrotra (1968) noticed that sulphate was good whereas metabisulphite, or persulphate was poor for growth of two Mucor sp. Agnihotri (1969) reported sulphate as best and sulphite as poorest sulphur source for growth and sclerotia production by Aspergillus niger. According to Singh and Tandon (1971) hyposulphite, thio-sulphate and sulphate were satisfactory sources for nutrition of Alternaria tenuis. Babich and Stotzky (1978) reported that though differential sensitivities were noted among the tested bacteria and fungi, yet bisulphite was always more inhibitory than sulphite. Similarly Mejstrik (1978) found that even 84 ug.m^3 sulphur dioxide was inhibitory to growth of Flammulina velutipes, Nematoloma fasciculare and Pleurotus ostreatus.

On the other hand, Kurek (1979) showed that Rhodotorula was able to utilize thiosulphate or sulphite though the enzymes responsible for oxidation of both compounds were non-inducible.

Soldatini et al., (1978) showed that below 1 mM, sulphite enhanced growth of Chlorella vulgaris coupled

with increased carbon fixation; a phenomenon that was also recorded by Stamm (1980) at concentrations below 0.3 mM using Chlorella vulgaris, Scenedesmus and Chlamydomonas reinhardtii.

The results of table 1 further show that smaller doses of thiosulphate or metabisulphite stimulated growth of Phormidium angustissimum in sulphate media whereas smaller doses of sulphate, slightly, if at all, affected growth in either thiosulphate or metabisulphite media. This confirms the prevalence of a dithionate pathway for sulphur metabolism similar to that recorded by Wainwright and Killham (1980) for Fusarium solani. Similarly, sulphate - sulphite mixtures stimulated but sulphate - persulphate mixtures seemed without effect. Since under all conditions, the pH changes, during 10 days of growth, were minor (pH 6.8 to 7.2) one would not expect pH fluctuations as one of the main factors causing variation in absorption rates, inhibition and/or toxicity by these sulphur sources. On the contrary, sulphate and sulphite seemed antagonising each other but sulphate and persulphate were not.

Since Garsed and Read (1977) noticed that sulphur dioxide was rapidly transferred to sulphate; in soya bean leaves, in the light, but substantial quantities of sulphite were present in the dark, one may suggest that the presence of sulphate, in sulphite media, prevented the latter's oxidation by light, thus the observed stimulatory effect on growth, being still with its comparatively higher redox potential. The inert effect of sulphate and/or persulphate might be attributed to their ^{highest} maximum state of oxidation thus devoid of any lone pairs or being with least or even no redox.

In this connection it may be mentioned that Cowling and Lockyer (1977) reported that although sulphur dioxide could replace sulphate as sulphur source for rye grass yet it had no effect on yield when coupled with sulphate. Soldatini et al. (1978) proved the preferential absorption of sulphite from its mixtures with sulphate by Chlorella vulgaris. Hodson and Schiff (1971) showed that thiosulphate was the major product formed by Chlorella, Euglena, Chlamydomonas, Anacystis, Escherichia, Salmonella and Baker's yeast whereas sulphite and sulphide were absent. In the meantime, oxidation of sulphite to

APS (adenosine 5-phosphosulphate) by bacteria had been demonstrated by Aminuddin (1979). Burnell and Whatley (1980) showed that sulphate uptake by Paracoccus denitrificans was non-competitively inhibited by sulphide and sulphite and competitively by thiosulphate. This suggested that sulphide and sulphite may function as feedback inhibitors of sulphate uptake whereas structural similarity between sulphate and thiosulphate suggested competition of both ions for the binding sites on the sulphate carrier protein.

Pasternak et al. (1965), Hodson and Schiff (1971) proposed that the enzymes of sulphate assimilation, in bacteria, were subject to differential rather than co-ordinate repression.

Table 2 further shows that although thiosulphate induced the maximum dry weight ~~gain~~ yet the chlorophyll a content per unit dry biomass was far less than the other treatments that seemed without effect compared with sulphur-starved samples, except sulphate that had a tendency to lower chlorophyll a level. This might be attributed to the stimulatory effect of thiosulphate on the other vital metabolic activities than chlorophyll a formation.

Still delayed senescence and/or wear and tear of chlorophyll might account for the stimulated gain of dry weight.

The presence of sulphate in thiosulphate media did not antagonise the inhibitory effects of the latter ion on chlorophyll a accumulation except at 20 % thiosulphate where a slight increase (below 100 % sulphate content) was observed, an indication that repression of chlorophyll a synthesis was not competitive. Sulphite or metabisulphite did not exert any competitive effect on chlorophyll a synthesis in sulphate media but persulphate was very effective, at its lower than higher levels, as observed from the larger increments in chlorophyll a in such mixed sulphur media that declined with increased persulphate content.

The results, recorded in table 3. further show that dithionates seemed better stimulators for carotenoid accumulation than monothionates; a phenomenon that was highly apparent at the higher than lower states of oxidation of the ion i.e. with decreased redox potential. This is quite obvious since persulphate induced the largest gain followed by metabisulphite then thiosulphate that hardly affected carotenoid content compared with

monothionates or sulphur deficient samples though sulphite tended to lower carotenoid content.

The presence of sulphate in metabisulphite media slightly counteracted the stimulatory effect of the ion whereas its presence in sulphite media slightly stimulated carotenoid formation; a phenomenon that was remarkably increased in thiosulphate or persulphate media.

It should be recalled that Haertel and Miklan - Grassl (1974) reported that sulphurous acid reduced water permeability of cells even at $10^{-5}N$. Still long term exposure to sublethal doses of sulphur dioxide induced considerable shrinkage of cell nuclei in apical meristems of Lemna minor (Stoeckli et al., 1975). Similarly, Matsushima et al. (1975) showed that 1-2 ppm sulphur dioxide exposure of 3-years old Zelkova serrata potted plants for 8 hours/day caused severe plasmolysis and destructed ribosomes and cytoplasm without severe destruction of the lamellar structure, within the leaf cells. Uhrling (1978) reported that, apart from disintegration of the circular chloroplasts of Petunia hybrida, sulphur dioxide induced plasmolysis, aggregation of cytoplasmic contents and disintegration of nuclei.

Compared with other results, we may conclude that, within 26 p.p.m. dose or 0 - 8 mM level, Phormidium angustissimum is more tolerable to the tested inorganic sulphur sources, including sulphite ion; compared to higher plants or other algae. This deduction is confirmed by the fact that with sulphite feeding, chlorophyll a accumulation was enhanced without affecting dry weight or carotenoid content.

It should be mentioned that Miyake et al. (1974) claimed that 3, 10 or 30 p.p.m. sulphur dioxide delayed the maturation of tobacco leaves. Wodzinski and Alexander (1978) showed that the photosynthetic activity of Anabaena flusagua and Chlamydomonas reinhardtii was totally inhibited, within 24 hours exposure to 1 p.p.m. sulphur dioxide when the pH of the medium was acidic. Maintaining the medium at pH 6 nullified such effect. They deduced that the decrease of the pH was not the sole cause of inhibition of photosynthetic activity. Ichikawa et al. (1974) showed a decrease in chlorophyll content when sulphur - fed kidney beans were exposed to small doses of sulphur dioxide. Hirai and Tsubota (1975) noticed that chlorophyll a was more sensitive to degradation than chlorophyll b when leaves were fed with sodium sulphite.

It should be recalled that the increased chlorophyll a content in persulphate - fed samples coupled with minor, if at all, effects on biomass yield indicates that this ion stimulated chlorophyll synthesis; more prominently when mixed with smaller doses of sulphate, accompanied by lowering photosynthetic activity. Still deviations in rate of respiration and/or metabolic activities may be effective.

In this connection, Ushizima et al. (1975) mentioned that carbon dioxide intake began to reduce as soon as exposure of sunflower plants to sulphur dioxide was begun. Ushijima et al. (1976) concluded that sulphur dioxide directly inhibited carbon dioxide fixing systems of sunflower; a phenomenon that was confirmed by other investigators working with other plants (Taniyama, 1976 a, b; Black and Unsworth, 1979). Ziegler (1972, 1973) showed that sulphite ion non-competitively inhibited ribulose 1: 5 diphosphate and P E P carboxylases. The latter enzyme as well as malic dehydrogenase were not affected by 10 mM sulphite. Spedding et al. (1980), proved that spinach chloroplasts absorbed sulphate ions, at a constant rate, within pH 5 - 8 range. Similarly

sulphite ion was absorbed but the rate was doubled with lowering the pH to 5.

Analysis of media and biomass for nitrogen components revealed that total nitrate disappearance from the media was indifferent to sulphur sources except thiosulphate that favoured a slight inhibition that was abolished by mixing with sulphate; particularly at the larger doses of either ions. Mixing sulphate with bisulphite hardly affected whereas coupling sulphate with sulphite inhibited nitrate disappearances. On the other hand, sulphate enhanced nitrate disappearance from persulphate media.

These observation might suggest that thiosulphate slightly lowered the permeability of Phormidium angustissimum cells towards nitrate uptake, a phenomenon that was antagonised by sulphate ion. Sulphate ion did not affect the permeability to nitrate ions in metabisulphite media but was inhibitory, in sulphite media, and stimulatory, in persulphate media; an indication that the similarity or dissimilarity in structure and/or redox potential of the various sulphur ions participated in the response of permeability towards their mixture.

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Irrespective of such fluctuations, the ratio of secreted nitrogen was less in thiosulphate media compared with other sulphur sources where it was almost similar to that by sulphur-starved biomass. This indicates better utilization of the absorbed nitrate from thiosulphate media compared with the other sulphur sources. Such increased ratio of nitrogen gain within the algal biomass was not counteracted by the presence of sulphate ion in the media. On the other hand, the increased ratio of nitrogen secretion, in mixed media of sulphate with other sources, indicates a lower nitrogen gain by the biomass i.e. deleterious effect of increased permeability; that was more prominent in persulphate media.

Apart from these deductions, sulphite seemed to inhibit whereas thiosulphate or metabisulphite stimulated nitrite reductase compared with sulphur deficient or sulphate-fed samples. Also persulphate had no significant effect. This is apparent from the fluctuations of nitrite content of the various media. Furthermore, the presence of sulphate in thiosulphate, metabisulphite or sulphite media stimulated amino nitrogen secretion; particularly at its larger percentages, a phenomenon that

reversed when sulphate was added to persulphate media. Such fluctuations were majorly on the expense of the other soluble nitrogen ratio, in the media, whereas the peptide nitrogen ratio was remarkably lowered with the larger proportion of persulphate. This indicates the differential permeability to the various nitrogen components when sulphate was mixed with the other sulphur sources (competitive and non competitive effects).

It should be mentioned that Tomonaga (1967) noticed that during sulphur deficiency, the synthesis of protease, by Aspergillus niger occurred preferentially but that of cellular proteins (including acid phosphatase) did not. According to Sakaguchi (1972), nitrate reductase was accelerated by 10^{-3} - 10^{-5} M sulphite ion but higher concentrations were inhibitory. Sulphite also inhibited formation of methemoglobin when rabbit erythrocytes were suspended in nitrite solutions.

Yokota et al. (1972) also noticed that protease, amylase and alkaline phosphomonoesterase were inhibited by sulphite, bisulphite, thiosulphate and metabisulphite whereas the microbial taka diastase was not affected; a phenomenon that was also reported by Malhotra (1980)

for acid phosphatase of Jack bean and by Rabe and Kreeb (1980) for glucose-6-phosphate dehydrogenase, isocitric dehydrogenase, glutamic dehydrogenase, alanine amino transferase and asparatic amino transferase of Medicago sativa and Viola tricolor, when these plants were exposed to large doses of sulphur dioxide. The last authors noticed that the crude enzymes from plant extracts were less affected by sodium sulphite than pure preparations. Jaeger (1975, 1977) noticed that the inhibitory effects of sulphur dioxide on GOT and GPT activities, in higher plants, was always accompanied by stimulated reductive amination (enhanced glutamic dehydrogenase activity).

Analysis of algal biomass indicated that thiosulphate enhanced nitrate reductase as could be observed from its lower level and percentage. This was accompanied by increased ratio of other soluble on the expense of peptide with slight reduction in the ratio of protein nitrogen. This shows the inhibitory effects of thiosulphate on peptide formation without affecting protein and other insoluble nitrogen (cell wall and nucleoprotein nitrogen). Similarly, metabisulphite, though slightly inhibited nitrate reduction yet it enhanced other insoluble nitrogen (cell wall and nucleoprotein nitrogen) on the expense of protein and other

soluble without affecting the ratio of soluble to insoluble components. Such tendency towards increased cell wall and nucleoprotein nitrogen might suggest a mean of resistance against metabisulphite toxicity that appear in inhibited protein synthesis; a phenomenon that was more apparent with persulphate treatment that did not affect protein synthesis and thus leading to a higher ratio of insoluble than soluble nitrogen components.

On the other hand, sulphite lowered the ratio of other soluble and insoluble nitrogen with a concomitant increase in peptide with little, if at all, effect on protein nitrogen. This indicates that ^e low resistance of the algal wall, coupled with accumulation of most of the cell nitrogen in the form of peptides.

Such particularities of the various sulphur sources were not changed by mixing with various ratios of sulphate ion; an indication of non-competitive effect.

In this connection it may be mentioned that Jaeger (1977) noticed that spruce needle and pea plants, exposed to sulphur dioxide, synthesised increased amounts of glutamate, stimulating the formation of glutamine and other amino acids of the glutamate family. The enhanced

formation of acidic amides may be a N-detoxification process. Shapiro (1977) explained the specific mutagenesis in microorganisms, at high bisulphite concentration, to be likely due to deamination of cytosine.

The results of this investigation further show that compared with sulphate-fed or sulphur deficient samples, thiosulphate, metabisulphite or persulphate inhibited phosphorus accumulation in the algal biomass whereas sulphite seemed without effect; a phenomenon that was partially or completely alleviated by addition of sulphate ion to such media; more prominently at larger than smaller doses. The presence of sulphate in sulphite media almost had no effect. This adds further evidence in support of the effect of these ions on the permeability of Phormidium cells.

Apart from these fluctuations, thiosulphate favoured a low inorganic phosphorus ratio with a concomitant increase in soluble organic ratio without affecting the insoluble components. Metabisulphite seemed without effect compared with sulphate whereas sulphite apparently inhibited soluble organic phosphorus with a parallel increase in DNA ratio. On the other hand, the increased organic phosphorus, in persulphate media, was accompanied

by reduced DNA ratio. Under all conditions, the presence of sulphate, in these media did not affect the ratio of the various phosphorus components; an indication of the non-competitive effect of such ions.

It is well known that the soluble organic phosphorus is majorly soluble nucleotides including coenzymes and ATP. The large ratio of such fraction denotes better energy supply i.e. active metabolism, particularly when RNA and/or DNA were not lowered. The latter condition, might lead, to inhibited metabolism and cell damage.

In this connection, it may be mentioned that Harvey and Legge (1979) noticed a drop in ATP content of Jack pine needles, that was furthered with increased level of sulphur dioxide in the atmosphere. Shapiro (1977) concluded that bisulphite had a number of reactions with nucleic acids and cellular molecules but it was not yet been demonstrated which was the responsible for the observed inhibited DNA synthesis.

SUMMARY

SUMMARY

The results of this investigation clearly establish the fact that growth of Phormidium angustissimum was not affected by absence of sulphur as a nutrient element. Thiosulphate seemed to be a better source of sulphur for maintaining maximum growth vigour of the organism by initiating maximum gain in dry weight, followed by metabisulphite whereas sulphite, sulphate or persulphate hardly affected growth though the latter source tended to be inhibitory compared to sulphur deficient samples.

Small doses of thiosulphate or metabisulphite stimulated growth of Phormidium angustissimum in sulphate media whereas smaller doses of sulphate, slightly, if at all, affected growth in thiosulphate or metabisulphite media. Similarly sulphate-sulphite mixtures stimulated but sulphate-persulphate mixtures seemed without effect.

Thiosulphate lowered chlorophyll a content whereas the other sulphur sources seemed without effect compared with sulphur deficient samples. The presence of sulphate in thiosulphate media did not antagonise the inhibitory effects of the latter ion on chlorophyll a accumulation. Sulphite or metabisulphite did not exert any competitive effect on chlorophyll a synthesis in sulphate media but persulphate was very effective.

Dithionates seemed better stimulators for carotenoid accumulation than monothionates. The presence of sulphate in metabisulphite media slightly counteracted the stimulatory effect of the ion whereas its presence in sulphite media slightly stimulated carotenoid formation; a phenomenon that was remarkably increased in thiosulphate or persulphate media.

Nitrate disappearance from the media was indifferent to sulphur sources except thiosulphate that favoured a slight inhibition that was abolished by mixing with sulphate; particularly at the larger doses of either ions. Mixing sulphate with bisulphite hardly affected whereas coupling sulphate with sulphite inhibited nitrate disappearances. On the other hand, sulphate enhanced nitrate disappearance from persulphate media.

Sulphite seemed to inhibit whereas thiosulphate or metabisulphite stimulated nitrite reductase whereas persulphate had no effect. The presence of sulphate in thiosulphate, metabisulphite or sulphite media stimulated amino nitrogen secretion, a phenomenon that was reversed when sulphate was added to persulphate media.

Analysis of algal biomass showed that thiosulphate enhanced nitrate reductase. This was accompanied by in-

creased ratio of other soluble on the expense of peptide and slight reduction in the ratio of protein nitrogen. Similarly metabisulphite slightly inhibited nitrate reductase but enhanced other insoluble nitrogen accumulation on the expense of protein and other soluble component; a phenomenon that repeated itself with persulphate though protein content was not affected. Sulphite lowered the ratio of other soluble and insoluble nitrogen coupled with increase in peptide content without affecting the proteins. Such particularities were not changed by mixing with various ratios of sulphate ion.

The results of this investigation further show that compared with sulphate-fed or sulphur deficient samples, thiosulphate, metabisulphite or persulphate inhibited phosphorus accumulation in the algal biomass whereas sulphite seemed without effect; a phenomenon that was partially or completely alleviated by addition of sulphate ion to such media; more prominently at larger than smaller doses. The presence of sulphate in sulphite media almost had no effect.

REFERENCES

REFERENCES

- AGNIHOTRI, V.P. (1969):
Some nutritional and environmental factors affecting growth and production of sclerotia by a strain of Aspergillus niger. Can. J. Microbiol. 15(8): 835-840.
- ALEXANDER, M.. (1961):
Introduction to Soil Microbiology". John Wiley and Sons, Inc. New York and London.
- AMINUDDIN, M. (1979a):
Oxidation of elemental sulphur by Thiobacillus denitrificans. Pertanika 2(1): 21-27.
- AMINUDDIN, M. (1979b):
The oxidation of sulfite by adenosine-5-phosphosulfate reductase in Thiobacillus denitrificans. Pertanika 2(2): 118-124.
- ASHENDEN, T.W. (1978):
Growth reductions in Cocks foot (Dactylis glomerata L.) as a result of sulphur dioxide pollution. Environ. Pollut. 15(2), 161-166.
- ASHENDEN, T.W. (1979):
The effects of long term exposures to SO₂ and NO₂ pollution on the growth of Dactylis glomerata L. and Poa pratensis L. Environ. Pollut. 18(4): 249-258.

- BABICH, H. and STOTZKY, G. (1972):
Ecologic ramifications of air pollutions.
Soc. Auto. Eng. Trans. 81, (1955-1971).
- BABICH, H. and STOTZKY, G. (1974):
Air pollution and microbial ecology. Crist. Rev.
Environ. Control 4, 353-421.
- BABICH, H. and STOTZKY, G. (1978 a):
Atmospheric pollution:
Impacts on and interactions with Microbial Ecology.
Microbial Ecology by W. Loutit and J. Airmiles
Springer-Verlag. Berlin Heidelberg-New York.
- BABICH, H. and STOTZKY, G. (1978 b):
Influence of pH on inhibition of bacteria, fungi
and coliphages by bisulphite. Environ. Res. 15(3):
405-417.
- BALCHUM, O.J., DYBICKI, J. and MENECLY, G.R. (1960 a):
The dynamics of sulfur dioxide inhalation, absorption,
distribution and retention. Arch. Ind. Health, 21:
564-569.
- BALCHUM, O.J., DYBICKI, J. and MENECLY, G.R. (1960 b):
Pulmonary resistance and compliance with concurrent
radioactive sulfur distribution in dogs breathing
 $^{35}\text{SO}_2$. J. Appl. Physiol. 15:62-66.
- BERNER, R.A., (1970):
Sedimentary pyrite formation. Am. J. Sci., 268:
1-23.
- BLACK, V.J. and UNSWORTH, M.H. (1979):
A system for measuring effects of sulfur dioxide
on gas exchange of plants. J. Exp. Bot. 30 (114):
81-89.

BOZIAN, R.H., STOTZKY, G.: (1976):

Inhibition of nitrification in soil by sulphur dioxide, and the effect of kaolinite and monotorillonite on inhibition. Agron. Abstr. p.135.

BRESSAN, R.A., LLOYD, G.W. and PHILIP, F. (1978):

Mechanisms of resistance to sulfur dioxide in the cucurbitaceae. Plant Physiol. (Bethesda) 61(5): 761-767.

BUECHLEY, R.W., RIGGAN, W.B., HASSELBLAN, V. & VAN BRUGGEN, J.B. (1973):

SO₂ levels and perturbations in mortality. a study in the New York/ New Jersey metropolis. Arch. Environ. Health, 27: 134-137.

BURNELL, J.N. and WHATLEY, F.R. (1980):

Regulation of sulphur metabolism in Paracoccus denitrificans. J. Gen. Microbiol. (118) 73-78.

BURTON, J.O. and RILEY, J.P. (1954):

In "Modern Methods of Plant Analysis". Paech, K. and Tracey, M.V. (1955). Vol. 2, P. 370. Springer Verlag, Berlin.

CHANEY, A.L. and MARBACH, E.P. (1962):

Modified reagents for determination of urea and ammonia. J. Clin. Chem. 8, 130-132.

COWLING, D.W. and LOCKYER, D.R. (1977):

The effect of sulphur dioxide on Lolium perenne L.
Grown at different levels of sulphur and nitrogen
nutrition. J. Exp. Bot. 29 (108), 257-266.

EL-AYOUTY, E.Y. (1966):

Systematik und stickstoffbindung einiger. Blaualgen
in Lehmboden aus einem humiden and einem Semiariden
Gebiet. Naturwissenschaftliche Fakultät der Justus
Liebig Universität zu Giessen.

ELORANTA, V. and ELORANTA, P. (1980):

Algal assays on water receiving sulfite and sulfate
cellulose effluents. Ann. Bot. Fenn. 17(1) 26-34.

FAWCETT, J.K. and SCOTT, J.E. (1960):

A rapid and precise method for the determination of
urea. J. Clin. Pathol. 13, 156-159.

FERGUSON, P., LEE, J.A. and BELL, J.N.B. (1978):

Effects of sulphur pollutants on the growth of
Sphagnum species. Environ. Pollut. 151-162.

FOGG, G.E. (1942):

Studies on nitrogen fixation by blue green algae.

I. Nitrogen Fixation by Anabaena cylindrica.

Lemm. J. Expt. Biol. 19, 78.

FRIEND, J.P. (1973):

The global sulphur cycle. In: Rasool, S.I. (ed.)
Chemistry of the Atmosphere. Plenum Press, New
York-London, 177-201.

GARSED, S.G. and READ, D.J. (1977):

The uptake and metabolism of $^{35}\text{SO}_2$ in plants of differing sensitivity to sulphur dioxide. Environ Pollut. 13 (3) 173-186.

GOLDHABER, M.B. and KAPLAN, I.R.; (1974):

The sulfur cycle.

In : E.D. Goldberg (Editor), The Sea. Wiley-Interscience, New York, NY, Vol. 5 pp. 569-655.

GRANAT, L.; RODHE, H. and HALLBERG, R.O. (1976):

The global sulphur cycle. In Svensson, B.H. and Soderlund, R. Leds, Nitrogen, Phosphorus and sulphur-global cycles. Scope Report 7. Ecol. Bull. StO.

GRANT, I.F.; BANCROFT, K. and ALEXANDER, M. (1979):

Effect of sulphur dioxide and bisulphite on heterotrophic activity in an acid soil. Applied and Environmental Microbiology, p. 78-83.

GUSEV, M.V.; NIKITINA, K.A.; GORSKAYA, N.V.; BELOGUROVA, N.G.

and MOCHALOV, V.V. (1979):

Blooming and destruction of cyanobacteria in the reservoir of a sulphur spring in staraya Matsesta, Russian SFSR, USSR. Mikrobiologiya 48(6): 1093-1101.

HAERTEL, O. and MIKLAN-GRASSL, S. (1974):

The effect of sulphur dioxide in the form of sulphurous acid on plant cells. Phytion (Austria), 16 (1-4), 81-99.

HALLGREN, J.E., and HUSS, K. (1975):

X Effect of sulphur dioxide on photosynthesis and
nitrogen fixation. *Physiol. Plant* . 34: 171-176.

HAMPP, R.; SPEEDING, D.J.; ZIEGLER, I. and ZIEGLER, H. (1980):

7 The efflux of inorganic sulphur from spinach
(*Spinacia oleracea*) Chloroplasts. *Z.Pflanzenphysiol.*
99(2): 113-120.

HANSON, M-A. and MARZLUE, G-A. (1973):

Regulation of a sulfur-controlled protease in
Neurospora crassa. *J.Bacteriol.* 116(2): 785-789.

HARVEY, G-W. and LEGGE, A-H. (1979):

X The effect of sulfur dioxide upon the metabolic
level of adenosine triphosphate. *Can. J.Bot.* 57(7):
759-764.

HEAGLE, A.S. (1973 a):

Interactions between air pollutants and plant
parasites. *Ann. Rev. Phytopathol.* 11, 365-388.

HEAGLE, A.S. (1973 b):

Response of three obligate parasites to ozone.
Environ. Pollut. 9(2): 91-95.

HIRAI, T. and TSUBOTA, T. (1975):

The effect of sodium sulphite on roadside tree
leaves. *Kinki Univ. Pollut. Res. Inst. Rep.*, vol.
3: 111-118.

HODSON, R.C. and SCHIFF, J.A. (1971):

Studies of sulfate utilization by algae. 8. The ubiquity of sulfate reduction to thiosulfate. Plant Physiol. 47: 296-299.

ICHIKAWA, N.; HASEBE, T.; and TAKAO, K. (1974-1975):

Effects of sulfur dioxide contact on high class kidney bean plants grown with different amounts of sulfur. Japan Soil Sci. Manure Hokkaido Branch Meet, p. 16.

JACO, V. (1977):

Rice sensitivity to microbial sulphur. Conostom. Ser. Biol. 12(2): 97-100.

JAEGER, H.J. (1975):

Effect of sulphur dioxide-fumigation on the activity of enzymes of the amino acid metabolism and the free amino acid contents in plants of different resistance. J. Pflanzenkrankh. Pflanzenschutz, 82(3): 139-148.

JAEGER, H.J. (1977 a):

Physiological and biochemical actions of sulphur dioxide on plants. Phyton (Horn) 18(1/2) 85-94.

JAEGER, H.J. (1977 b):

Effect of phytotoxic air pollution on enzymatic activities and reactions. Angew Bot. 51(1/2): 1-8.

JORGENSEN, B.B. and FENSHEL, T. (1974):

The sulphur cycle of a marine sediment model system.
Mar. Biol. 24: 189-201.

KAPLAN, I.R.; EMERY, K.O. and RITTENBERG, S.C. (1963):

The distribution and isotopic abundance of sulphur
in recent marine sediments of Southern California
Geochim. Cosmochim. Acta 27: 297-331.

KELLY, D.P. (1968):

Biochemistry of oxidation of inorganic sulfur
compounds by microorganisms. Aust. J. Sci. 31(5),
165-173.

KHVOLES, A.G. and KOROBKO, Y.A. (1977):

The action of dimethyl sulfate on the reproductive
system of the loach. Misgurnus fossilis (L.) Vopr.
Ikhtiol. 17(2): 371-373.

KOMMUNARSKAYA, A.D. (1970):

³⁵S-induced variability of Penicillium nigricans
producing griseofulvin. Antibiotiki 15(3): 216-218.

KORD, M. (1981):

Response of crop plants to pollution with sulphur
dioxide. Ph.D. Thesis, Botany Department, Facu-
lty of Science, (Cairo University).

KUMAR, V.; and SINGH, M., (1980):

Sulphur, Phosphorus and molybdenum
interactions in relation to growth, uptake and
utilization of sulphur in soy bean (Glycine max).
Soil Sci. 129(5): 297-304.

KUREK, E. (1979):

Oxidation of inorganic sulphur compounds by yeast.
Acta Microbiol. Pol. 28(2): 169-172.

LANDESMAN, J.; DUNCAN, D.W. and WALDEN, C.C. (1966):

Oxidation of inorganic compounds by washed cell
suspension of Thiobacillus ferrooxidans. (British
Columbia Res. Council, Vancouver) J. Microbiol.
12(5) 957-964.

LAURENCE, J.A. (1979):

Response of maize and wheat to sulfur dioxide.
Plant Dis. Rep. 63(6): 468-471.

LOVE, L.G. (1969):

Sulphides of metals in recent sediments.
In: Sedimentary Ores: Ancient and Modern (revised
edn.) Proc. 15th Int. Univ. Geol. Congr. (1967).
Dept. of Geol., Univ. Leicester, Gt. Britain, p.p.
31-60.

LOWRY, O.H.; ROSENBROUGH, J.; FAN, A.C. and RANDAL, R.J. (1951):

Protein measurement with Folin phenol reagent.
J. Biol. Chem. 193, 265-275.

MAIMOUD, S.A.Z., EL-DAMATY, A.A.; METWALLY, A.I. and YANNI,
Y.G. (1977):

Effect of physico-chemical factors on total micro-
bial flora and sulphate-reducing bacteria in water-
logged soils. Egypt. J. Microbiol., 12,(1-2)
p.p. 1-14.

MALHOTRA, S.S. and KHAN, A.A. (1980):

Effects of sulfur dioxide and other air pollutants on acid phosphatase (EC. 3.1.3.2) activity in pine (Pinus banksiana) seedling. Biochem physiol. Pflanz. (BPP) 175 (3): 228-236.

MARKOWSKI, A.; GRZESIAK, S. and SCHRAMMEL, M. (1975):

Susceptibility of six species of cultivated plants to sulphur dioxide under optimum soil moisture and drought conditions. Bull. Acad. Polon. Sci. Ser. Sci. Biol., 22(12): 889-898.

MASOHARA, O. and KANEO, M. (1974):

Studies on the effect of air pollution on crops, Part I. Effects of gaseous sulfur dioxide and ozone on the occurrence of symptoms of injuries on vegetables. Bull. Osaka Prefect. Agric. Res. Cent. 11, 151-157.

MATSUSHIMA, F.; ODAIRA, T.; SAWADA, T. and NOUCHI, I. (1975):

Effects of ozone, ethylene, nitrogen dioxide and sulphur dioxide on the microstructure of leaves of Zelkova serrata. J. Japan. Soc. Air Pollution 10(4): 454.

MAYUMI, Y. and YAMAZOE, F. (1971):

On the distribution and states of sulfur in the plant body damaged by sulfur dioxide. Japan Soc. Soil Sci. Manure Meet., Osaka, pp. 170.

MEHROTRA, B.R. (1968):

Sulphur requirements of 2 species of Mucor. Flora
Abt. A Physiol. Biochem. (Jena) 159 (5): 403-407.

MEJSTRIK, V. (1978):

Influence of sulphur dioxide on the growth of some
fungal species. Zentralb. Bakteriologie parasitenkd.
Infektion skr. Hyg. Zweite Naturwiss. Abt. Mikrob-
iol. Landwirtsch. Technol. Umweltschutzes 133(5):
464-467.

METZNER, H., RAU, H. and SENGER, H. (1965):

Untersuchungen zur Synchronisierbarkeit ein-
zelner pigmenten^pangel-Mutanten von Chlorella.
Planta, 65, 186.

MIYAKE, Y. and UNO, Y. (1973):

The effect of sulphur dioxide at concentrations of
0, 14 and 30 ppm on tobacco plants. Bull. Utsunomiya
Tob. Exp. Stn. No. 12.

MIYAKE, Y.; UNO, Y.; TAKANASHI, N. and AOKI, M. (1974):

On the damage of tobacco leaves due to sulfurous acid
gas 2. The change of leaf components due to
the damage due to the gas. Japan. Soc. Soil. Sci.
Manure Ann. Meet., 20th. pp. 116.

MONHEIMER, R.H. (1979):

Effect of cysteine and methionine on sulfate uptake
and primary productivity by axenic algal cultures
and lake microplankton communities J. Phycol. 15(3):
284-288.

MOURIOUX, G. and DOUCE, R. (1979):

Carrier-mediated transport of sulfate through the envelope of spinach chloroplast. *Biochimie (Paris)* 61 (11/12), 1283-1292.

X *Tagu...*
NATALIA, B.; TERESA, W. and ELZBIETA, C. (1977):

Microorganisms as indices of environmental pollution by smelting industry. *Acta. Microbiologica polonica*. 26 (3): 301-308.

PAECH, K. and TRACEY, M.V. (1956):

Modern methods of plant analysis. Springer-Verlag, Berlin.

PASTERNAK, C.A., ELLIS, R.J.; JONES-MORTIMER, M.C. and

CRICHTON, C.E. (1965):

The control of sulphate reduction in bacteria. *Biochem. J.* (96) 270-275.

PINTER, I.I. and PROVASOLI, L. (1958):

In Singh, R.N. (1961), Role of Blue Green Algae in Nitrogen Economy of Indian Agriculture. Ind. Council Agr. Res. New Delhi.

PRINGSHEIM, E.G. (1951):

Methods of Cultivation of Algae. In Smith, G.M.: Manual of Phycology. 347. Chronica Botanica Co.

RABE, R. and KREEB, K.H. (1980):

Effects of SO₂ upon enzyme activity of plant leaves. *Z. Pflanzenphysiol.* 97 (3): 215-226.

RASMUSSEN, K.H.; TAHERI, M. and KABEL, R.L. (1975):

Global emissions and natural processes for removal of gaseous pollutants . Water Air Soil Pollut. 4, 33-64.

RICKARD, D.T. (1969):

The chemistry of iron sulphide formation at low temperatures. Stockholm Contribution to Geology, V: 20, pp. 67-95.

ROBINSON, E. and ROBBINS, R.C. (1972):

Emissions, concentrations and fate of gaseous atmospheric pollutants. In: Strauss, W. (ed.) Air Pollution Control, Vol. II. Wiley (Interscience), New York, 1-93.

RUSSEL, J.A. (1944):

Colorimetric detection of amino nitrogen. J. Biol. Chem. 56, 467.

SAKAGUCHI, O.; Chen, R-Y. and YOKOTA, K. (1972):

Effect of sulphite on nitrate reductase, nitroreductase, and methemoglobin formation. Jap. J. Hyg. 26(6): 492-497.

SAUNDERS, P.J.W. (1971):

Modification of the leaf surface and its environment by pollution. In: Ecology of Leaf Surface Microorganisms. Preece, T.F. and Dickinson, C.H. (ed.). New York: Academic Press, pp. 81-89.

SCHIMZ, K.L. (1980):

The effect of sulphite on the yeast, Saccharomyces cerevisiae. Arch Microbiol. 125 (½): 89-96.

SCHMIDT, G. and THANHAUSER, S.J. (1945):

A method for detection of deoxyribonucleic, ribonucleic acid and phosphorproteins in animal tissues. J. Biol. Chem. 161, 83.

SHAPIRO, R. (1977):

Genetic effects of bisulphite (Sulphur dioxide). Mutat. Res. 39 (2): 149-175.

SHIMAZAKI, K-I. and SUGAHARA, K. (1980):

Inhibition site of the electron transport system in lettuce (Lactua sativa cultivar Romaine) chloroplasts by fumigation of leaves with sulfur dioxide. Plant Cell Physiol. 21(1): 125-136.

SINGH, B.P., and TANDON, R.N. (1971):

Sulphur requirements of certain isolates of Alternaria tenuis Auct. Mycopathol. Mycol. Appl. 43(1): 97-101.

SINGH, M. and KUMAR, V. (1979):

Sulfur, phosphorus and molybdenum interaction on the concentration and uptake of molybdenum in soy bean plants (Glycine max). Soil Sci. 127(5): 307-312.

SKAKEEB M.A. (1975):

Untersuchungen zur Biochemie und Physiologie von cyanophyceen und farblosen Prokaryoten. Diss. Tübingen.BRD.

SMITH, J.J. and DAVIS, D.D. (1977):

The influence of needle age on sensitivity of Scotch pine to acute doses of sulphur dioxide. Plant Dis. Rep. 61 (10), 870-874.

SOLDATINI, G.F.; ZIEGLER, I. and ZIEGLER, H. (1978):

Sulfite: Preferential sulfur source and modifier of carbon dioxide fixation in Chlorella vulgaris. Planta (Berl.) 143(2), 225-232.

SPEDDING, D.J.; ZIEGLER, I.; HAMPP, R. and ZIEGLER, H. (1980 a):

Effect of PH on the uptake of sulfur-35-labelled sulfate, sulfite and sulphide by Chlorella vulgaris. Z.Pflanzenphysiol. 97(3), 205-214.

SPEDDING, D.J., ZIEGLER, I.; HAMPP, R. and ZIEGLER, H. (1980 b):

Effect of PH on the uptake of sulfur-35-from sulfate, sulfite and sulfide by isolated spinach chloroplasts. Z.Pflanzenphysiol. 96(4), 351-364.

SPRUGEL, D.G.; MILLER, J.E.; MULLER, R.N.; SMITH, H.J. and

XERIKOS, P.B. (1980):

Sulfur dioxide effects on yield and seed quality in field-grown soybean (Glycine max, cultivar wells). Phytopathology 70(12), 1129-1133.

STAMM, A. (1980):

The effects of sulfite on the growth and carbon dioxide fixation of unicellular green algae. Environ. Pollut., Ser. A 22(2): 91-99(Ger).

STARKEY, R.L. (1966):

Oxidation and reduction of sulfur compounds in soils. Soil Sci. 101 (4), 297-306.

STOECKLI, B.; BRAENDLE, R. and ERISHMANN, K.H. (1975):

Effects of low sulphur dioxide concentrations on the mitotic activity and on the cell cycle of duck weed (Lemna minor L.). German Experientia 21 (7): 795-796.

STOTZKY, G. and SCHENEK, S. (1976):

Volatile organic compounds and microorganisms. Crit. Rev. Microbiol. 4: 333-382.

SUBBA, R.N.S. (1977):

Soil Microorganisms and Plant Growth. Oxford and IBH Publishing Co. New Delhi 213-215.

SWEENEY, R.E. (1972):

Pyritization during diagenesis of marine sediments. Ph.D. Thesis, Dept. of Geology, Univ. California, Los Angeles, CA.

TANIYAMA, T. (1976 a):

Actual conditions of crop damage due to gaseous sulfur dioxide and studies on the mechanism of the damage. Crop. Sci. Soc. Jap. Lect. Meet, 16th, 189-190.

TANIYAMA, T. (1976 b):

Crop yield index and degree of pollution in field polluted by sulfur oxides, heavy metals from Annaka City. General Research Society for Environ. Science, Japan, p.10-11.

TOMONAGA, T. (1967):

Preferential synthesis of extracellular protease by Aspergillus niger in sulphur deficiency. J.Gen. Appl. Microbiol. 12(3), 267-276.

TUERK, R. and CHRIST, R. (1978):

Growth of lichens on oaks, Quercus robur, within the city of Salzburg, Austria, and water economy of some characteristic lichens. Phytol. (Horn) 18(314), 107-126.

UHRING, J. (1978):

Leaf anatomy of Petunia in relation to pollution damage. J. Am. Soc. Hortic. Sci. 103(1), 23-27.

USHIJIMA, T.; SATO, A.; FUKUSHIMA, Y. and TAZAKI, T. (1976):

Effects of sulfur dioxide on photosynthesis and rate of transpiration of plants. General Research Society for Environmental Science, Japan, p.48-49.

USHIZIMA, T.Y.FUKUSHIMA,J.; AOSHIMA, and TAZAKI,T. (1975):

Invisible injuries by sulfur dioxide to plants, centering on the effect to the photosynthetic rate of the leaf. Japanese Preprint, Japan Society for General Research, p. 89-90.

WAINWRIGHT, M. (1978):

A modified sulphur medium for the isolation of sulphur-oxidizing fungi.

Plant and Soil 49, 191-193.

WAINWRIGHT, M. (1979):

Microbial sulphur-oxidation in soils exposed to heavy atmospheric pollution. Soil Biol. Biochem. II(2), 95-98.

WAINWRIGHT, M. and KILLHAM, K. (1980):

Sulphur oxidation by Fusarium solani. Soil Biol. Biochem. 12(6), 555-558.

WATANABE, H. (1966):

Health effects of air pollution in Osaka City. I- Osaka life Hyg. Associ., 10: 147-157.

WODZINSKI, R.S.; LABEDA, D.P. and ALEXANDER, M. (1978):

Effects of low concentrations of bisulphite, sulphite and nitrite on microorganisms. Appl. Environ. Microbiol. 35: 718-723.

YOKOTA, K.; CHEN, R-Y., and SAKAGUCHI, O. (1972):

Effects of sulphur oxides on microbial hydrolytic enzymes and digestive enzymes. Jap. J. Hyg. 26(6), 481-485.

YOKOYAMA, E., YODER, R.E. and FRANK, N.R. (1971):

Distribution of ^{35}S in the blood and its excretion in urine of dogs exposed to $^{35}\text{SO}_2$. Arch. Environ. Health 22, 389-395.

ZIEGLER, I. (1972):

X The effect of sulphite on the activity of ribulose-1,5-diphosphate carboxylase in isolated spinach chloroplasts. *Planta*, (Berl.) 103, 155-163.

ZIEGLER, I. (1973):

Effect of sulphite on phosphoenol pyruvate carboxylate and malate formation in extracts of Zea mays. *Phytochemistry*: 12, 1027-1030.

بسم الله الرحمن الرحيم

ملخص لرسالة الدكتوراه

الطالبة / سكيمة توفيق عبد الغنى مدرس مساعد بقسم النبات

موضوع الرسالة

دور مصادر الكبريت في نمو وايض الفورميد يوم انجوستيسميوم

لقد اثبتت نتائج هذه الابحاث بوضوح ان طحلب الفورميد يوم انجوستيسميوم لا يتاثر نموه بعدم توفر الكبريت كعنصر غذائي . ويبدو ان الشيوكبريتات افضل مصدر للكبريت من حيث الحصول على اقصى نمو واكبر حجم للكائن وذلك عن طريق الزيادة الكبيرة في الوزن الجاف ويليها في ذلك ميتاثنائي الكبريتيت بينما الكبريتيت والكبريتات او الفوق كبريتات تتركز قليلا على النمو غير ان المركب الاخير يميل الى اعاقا النمو ويتضح ذلك عند المقارنة بالعينات التي تخلو من عنصر الكبريت .

وتنشط الجرعات القليلة من الشيوكبريتات او الميتاثنائي الكبريتيت النمو في طحلب الفورميد يوم في الوسط الغذائي الذي يحتوى على الكبريتات بينما يكون النمو ضعيفا او معدوما في الوسط الغذائي الذي يحتوى على الشيوكبريتات او الميتاثنائي الكبريتيت مع جرعات قليلة من الكبريتات . وكذلك ينشط الخليط من الكبريتات والكبريتيت النمو بينما لا يؤثر الخليط من الكبريتات والفوق كبريتات على النمو .

وتقلل الشيوكبريتات من المحتوى الكلورفيللى ١ . بينما يبدو ان المصادر الاخرى للكبريت ليس لها تاثير ملحوظ بمقارنتها بالعينات التي تخلو من عنصر الكبريت . وان وجود الكبريتات في الوسط الذي يحتوى على الشيوكبريتات لا يضاد اعاقا الايون الاخير لتراكم كلوروفيل ١ . لا يحدث الكبريتيت او الميتاثنائي الكبريتيت اى تاثير منافس على تمثيل كلوروفيل ١ في الوسط المحتوى على الكبريتات ولكن الفوق كبريتات لها تاثير كبير .

ويبدو ان ثنائي الشيونات من المنبهات المفضلة في تراكم الكاروتينات عن

احادى الشيونات • وان وجود الكبريتات فى الوسط المحتوى على الميثانائى الكبريتيت يعادل قليلا التأثير المنشط للايون بينما وجوده فى الوسط المحتوى على الكبريتيت ينشط قليلا تكوين الكاروتينات • وهذه الظاهرة تزداد وضوحا فى الوسط المحتوى على الشيوكبريتات او الفوق كبريتات •

ولا تختلف النترات عند اختفائها من الوسط باختلاف مصادرها الكبريت غير ان الشيوكبريتات تميل الى التثبيط قليلا الذى يتضح عند خلطها بالكبريتات وخاصة فى الجرعات الكبيرة لاحد الايونين • ويخلط الكبريتات وثنائى الكبريتيت يقلل تأثيرهما بينما ازدواج الكبريتات والكبريتيت يعوق اختفاء النترات • ومن ناحية اخرى فان وجود الكبريتات فى الوسط المحتوى على الفوق كبريتات يؤدى الى سرعة اختفاء النترات •

ويبدو ان الكبريتيت تعمل كمثبط بينما تنشط الشيوكبريتات او الميثاكبريتيت الانزيم المختزل للنيتريت ولا تحدث الفوق كبريتات اى تأثير وان وجود الكبريتات فى الوسط المحتوى على الشيوكبريتات والميثانائى الكبريتيت او الكبريتيت ينشط افراز النتروجين الامينى • وهذه الظاهرة تنعكس عند اضافة الكبريتات الى الوسط المحتوى على الفوق كبريتات •

وبتحليل كتلة الطحالب المتكونة اتضح ان الشيوكبريتات تنشط الانزيم المختزل للنيتريت • وهذه الظاهرة مصحوبة بالزيادة فى نسبة المواد الاخرى الذائبة على حساب الببتيدات والبعض الطفيف فى نسبة النتروجين البروتينى • وعلى نفس المنوال تعيق الميثانائى الكبريتيت نشاط الانزيم المختزل للنترات قليلا ولكنها تسرع من تراكم المواد النتروجينية الاخرى غير الذائبة على حساب البروتينات والمواد الاخرى الذائبة • وتكرر هذه الظاهرة نفسها فى وجود الفوق كبريتات مع ان المحتوى البروتينى لا يتأثر وتقلل الكبريتيت من نسبة المواد النتروجينية الاخرى الذائبة وغير الذائبة مع زيادة فى محتوى الببتيدى بدون التأثير على البروتينات • وهذه الخواص لا تتغير عند الخلط بنسب مختلفة من ايون الكبريتات •

وزياده على ذلك بان هذه النتائج تشير الى ان الشيوكبريتات والميتاثنائى الكبريتيت او الفوق كبريتات تعيق تراكم الفسفور فى الكتله الحيه للطحالب بمقارنتها بالعينات التى ينقصها او يتوفر لها الكبريتات بينما يبدو ان الكبريتيت ليس له تأثير . وهذه الظاهرة تتأثر جزئيا او كليا باضافة ايون الكبريتات للوسط بصورة اوضح عند استعمال جرعات كبيره عنها عند استعمال الجرعات الصغيره . وان وجود الكبريتات فى الوسط المحتوى على الكبريتيت لا يحدث تأثيرا .

دور مصادر الكبريت في نمو وايض طحلب الفورميدיום انجومستيميوم

رسالة مقدمة إلى

كلية العلوم - جامعة القاهرة

للحصول على درجة دكتوراه الفلسفة في النبات

(ميكروبيولوجى)

مقدمة من

سكينة توفيق عبد الغنى طنطاوى

ماجستير في العلوم (١٩٧٨)

كلية العلوم - جامعة القاهرة

قسم النبات

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