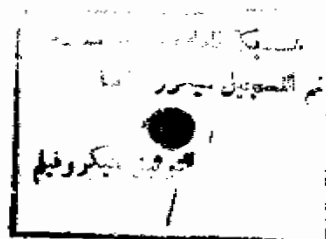


CYTOGENETIC RESPONSE OF SOME ECONOMIC PLANTS TO SODIUM SULPHITE

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INTRODUCTION

The term air pollution is a very broad one and includes, on the one hand, the spreading by air, of a large number of substances emitted into the atmosphere, and, on the other hand, a series of different chemical reactions between these substances, resulting in the formation of new pollutants.

Air pollution problems have existed for centuries but only in the last century and a half, a period of explosive scientific and technological advances, have these problems become severe. However, man's activities now attack the natural fresh air so severely that a pollution control which aims at abatement in several areas of the globe is necessary to prevent a threat to human health, animals, plants and ecosystem. Because of inter-regional and long range atmosphere transport, many national and international monitoring programs have been implemented.

Despite efforts to reduce emissions from particular sources and certain pollutants, both the amount of air pollution and the number of different contaminating substances have increased, due to the growth of the population and industrialization. Additional and co-ordinated activities to control air pollution are a subject of immediate concern to the industrialized countries but they should be of equal concern to other countries in the world (*Weber, 1982*).



Air pollution has serious effects damaging on both vegetation and horticultural crop production. Ozone is one of the most wide spread air pollutants. High ambient ozone concentrations contributed to the novel forest decline phenomena observed in Europe and North America (*Forschungsbeirat, 1989*) and to the decrease in productivity of crop plants (*Heck et al., 1988*). Data on the extensive physiological and biochemical effects of ozone on higher plants exist (*Heath, 1988; Sandermann et al., 1989*). SO_2 and NO_2 singly and in mixture had adverse effects on forest and agricultural crops (*Jones and Mansfield, 1982; Bell, 1982; Mansfield and Freer-Smith, 1981; Kord, 1981*).

Over 90% of primary pollutants, that is those which are emitted rather than produced by reaction in the atmosphere, originate from combustion process. These combustion processes include stationary sources such as industry and mobile source (vehicles) (*Jackson et al., 1989*).

Sulphur is a natural contaminant of coal, and is almost completely converted to sulphur oxide when coal is burned. About 95% of all sulphur oxide are in the form of sulphur dioxide (*EPA, 1980*). In the atmosphere, however, SO_2 is a precursor of highly destructive sulphates (SO_4), which are formed by the chemical addition of oxygen (O_2). SO_4 is not a stable compound, however, and in the presence of water it frequently forms

sulphuric acid ($H_2 \cdot SO_4$ FPA, 1980). The production of sulphuric acid gives rise to the acidification of rainwater and is part of the process leading to the formation of acid rain (EPA, 1979).

Acid rain is a major problem throughout the world, especially in Scandanavia, Canda, and the estern United States (EPA, 1980). It has achieved prominence in recent years because of the effects on forest trees, particularly in Scandinavia and West Germany (Jackson *et al.*, 1989).

The sensitivity of each individual plant to acid rain is different. The permeability dependent upon the wax layer of the cuticle, and the roughness of the leaf surface are very important factors (Arndt *et al.*, 1983; Evans, 1984). One of the possible reasons for the differing sensitivities of several species is probably the buffer state of the leaf (Ormrod, 1986).

The effect of acid rain on the soil is greatly influenced by the buffer capacity of the soil. A soil is very sensitive to acid rain if it contains no chalk and is rutrient free (Van Volten, 1984). The acid rain rinses out more nutrients than normal rain, and it can also rinse out aluminium ions and heavy metals so that toxification can take place. Indirectly, the rain can influence the microorganism content in the soil and, consequently, the degrading of the soil (Arndt *et al.*, 1983).

Sulphur dioxide may easily be oxidised in air to SO_3 , and both compounds have destructive environmental impacts. Oxides of sulphur may easily be washed by rainfall from the atmosphere as sulphite salts and are oxidised to sulphates (*Cotton, 1976*).

Sulphur trioxide is also emitted in industrial flue gases but the amounts are very low. It is therefore of much less importance as a primary pollutant even though it is more active than SO_2 . It has strong affinity for water. The hydrate of SO_3 is sulphuric acid or it hydrates to sulphurous acid (H_2SO_3 *Jackson et al., 1989*).



Although sulphurous acid does not exist, two series of salts, the bisulphites containing HSO_3^- and the sulphites containing SO_3^{2-} are well known. The SO_3^{2-} in crystals is pyramidal. Only the water-soluble alkali sulphites and bisulphites are commonly encountered (*Sitting, 1975*).

The most dramatic evidence of the adverse effect of air pollution come from the action of SO_2 , H_2SO_3 , H_2SO_4 , metal and other materials may be corroded or degraded especially when moisture is present. (*Cotton, 1976*).

The biochemical aspects of sulphite effects are well known. In aqueous solution sulphite is oxidised to sulphate (*Timson, 1973*) a process considerably retarded by glucose (*Roy and Trudinger, 1970*). Both sulphite and bisulphite anions are available in solution for reaction with cell constituents (*Yang, 1970*). Sulphite inhibits the activity of dehydrogenases, — amylases (*Yokota et al., 1972*) and AT pase as well as that of a variety of cations including sodium, potassium and magnesium (*Lacombe et al., 1976*).

A wide range of microorganisms was tested to determine their sensitivity to low concentrations of bisulphite - sulphite and nitrite, solubility products of SO_2 and NO_2 , respectively. Photosynthesis by blue-green algae was more strongly inhibited by 0.1 mM bisulphite-sulphite and 1 mM nitrite at PH 6.0 than photosynthesis by eucaryotic algae and respiration of bacteria, fugi, and protozoa. At PH 7.7, blue-green algae were still more sensitive than eucaryotic algae but the toxicity decreased as the PH increased (*Wodzinski et al., 1978*).

Liu et al., 1980, found that segments of wheat seedlings produced ethane production was proportional to NaHSO_3 concentration. At the higher Na HSO_3 conc. chlorophyll was decomposed.

The most readily interpretable results of bisulphite mutagenesis were obtained in the VII system of T_4 phage (*Summers et al., 1971*). Phage articles were allowed to react at pH 5.0, for times up 4 hrs., in bisulphite

concentrations up to 0.9 M. No alkaline incubation was used to decompose bisulphite adducts. However, phage T₄ contains 5-hydroxymethylcytosine in place of cytosine. The properties of the final 5-hydroxymethyluracil adducts with bisulphite would probably resemble those of thymine, and be relatively unstable in neutral growth media. Both inactivation and mutagenesis were observed and were proportional to bisulphite concentration and length of treatment. Control buffers caused neither mutation nor inactivation. Complete GC to AT mutogenic specificity was observed. Only mutants reverted by hydroxylamine were reverted by bisulphite, while frameshifts, and mutants containing AT sites, did not respond. Bisulphite was termed a moderately strong mutagen in this system.

Clark (1953) reported that 0.01 M bisulphite presumably neutral PH, was observed to be particularly active in promoting mutations to streptomycin resistance in *Micrococcus aureus*. A killing effect by 0.01 M bisulphite, at PH below 5, was reported for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *E.Coli* (*Richards and Reary, 1972*). Strains deficient in DNA polymerase were reported to be more sensitive than the wild type.

In higher plants, using aqueous bisulphite solution at concentrations above about 0.001 M, the mitotic index was depressed in the root meristems of *Vicia faba* and DNA synthesis was inhibited (*Brandle and*

Erismian, 1973, Njagi and Gopalan, 1982). Concentrations above 0.002 M impaired DNA synthesis and normal metabolic development in synchronous cultures of *Chlorella pyrenoidosa*. On the other hand, 0.002 to 0.004 M bisulphite, applied to germinating onion bulb root tips, exerted a protective effect (to 30%) against chromosome deletions and interchanges caused by x-rays (*Riley, 1957*).

Kak and Kaul (1979) found that, treatment of barley grains with 7 and 10 mM solutions of sodium bisulphite at 30°C for 6 hrs., caused up to 40% reduction in seedling height and mutation rates of 17.0 and 24.1% respectively, in the M_1 and 0.8 and 2.3% respectively, in the M_2 . The mutation included albino, viridis, and alboviridis.

Singh (1983) reported that, soaking of barley grains in 3, 6, 9 and 12 mM solutions of sodium bisulphite for 18 hrs before planting in the field, caused increase in the M_1 , seedling injury from 13.1 to 21.1% and spike sterility from 11.6 to 18.3% as the concentration of sodium bisulphite increased from 3 to 12 mM. In the M_2 , the frequency of chlorophyll deficient seedlings almost doubled (1.61 - 3.06 per 100 seedlings) with the same rise in bisulphite concentration.

Timson (1973) confirmed that 0.01 M bisulphite prevented mitosis in human lymphocytes, and even 0.0001 M reduced it by half. Again the early stages of mitosis, and perhaps DNA synthesis, were the most

vulnerable. Using mammalian cell cultures, low concentrations (less than 0.01 M) of bisulphite inhibited DNA synthesis, mitosis, and growth, and caused chromosomal abnormalities. Such effects were not observed when animals themselves were treated (*Shapiro, 1977*).

In vitro studies on the maturation of mammalian oocytes were conducted by *Jagiello et al. (1975)*. With mouse cells, some inhibition of entry into meiosis was observed at concentrations above 10^{-4} M bisulphite. Fuzziness of chromosomes was observed at concentrations below 0.001 M and occurred heavily at about 0.005 M. In cow oocytes a concentration of 0.0025 M produced various types of chromosome damage, and inhibition of meiosis. It was pointed out that clumping and fuzziness would lead to cell death, but that fragmentation observed could lead to transmissible disorders. Despite their failure to observe these effects *in vivo*, bisulphite was considered to be a potential genetic hazard.

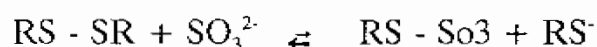
It is known that, sulphite and bisulphite may gain entry by inhalation as SO_2 , or by ingestion extensively as preservatives in foods, beverages and pharmaceuticals as sulphite and bisulphite salts (*Shapiro, 1977*). Bisulphite in a concentration of 0.01 M to 0.001 M inhibited the growth of microorganisms (*Chichester and Tanner, 1972*). This property is responsible for its use in wines, which is a principal source of human exposure. Lower

concentrations of bisulphite are needed at acidic than at neutral PH (*Cruess and Irish, 1932; Chichester and Tanner, 1972*). In this respect it may be mentioned that addition of sodium sulphite or sodium bisulphite to coffee inactivated the mutagenicity of coffee made in the ordinary way (*Suwa et al., 1982*).

Last et al. (1980) observed slight edema of the lungs of rats immediately following essentially continuous exposure for 72 hrs. to 1, 5, and 15 mg/m³ of sodium sulphite aerosols with mass median aerodynamic diameter (MMAD) of approximately 1.0 μ m. Edema was detected by an increase in lung wet to dry weight ratios.

Cohen and Fridovich (1971) reported that the principal mammalian defense against ingested or inhaled bisulphite is sulphite oxidase, which converts it to sulphate. This enzyme is molybdenum-containing protein, whose natural function is to oxidize endogenous sulphite (produced in the metabolism of sulphur-containing amino acids) to sulphate (*Cohen et al., 1973*). It is present in mammals, plants, and bacteria. In mammals the enzyme is distributed among a number of organs; liver is the principal site of activity, followed by kidney, with activity present also in the intestines, heart and lungs. Within the cell, the enzyme is present in the mitochondria.

Sulphite oxidase-deficient rodent models have been used to investigate the metabolism and toxicity of sulphite (*Gunnison et al., 1981 a, b; Dulak et al., 1984; Renner and Wever, 1983*). In these models, decreased activity of tissue sulphite oxidase resulted in higher in Vivo concentrations of sulphite due to accumulation from endogenous sources (i.e., catabolism of S-containing amino acids, and or from exogenous sources (e.g. ingestion of sulphite salts). Under these conditions, sulphite is much more likely to react with target molecules in various tissues. One category of such target molecules is those containing exposed disulphide bonds, for example, cystine and albumin. Sulphite reacts with the lysates of these susceptible disulphide bonds producing an S-sulphonate group (RS-SO₃⁻) attached to one sulphur atom and leaving the other sulphur atom in the reduced, thiol state (Sulphitolysis) (*Gunnison et al., 1987*).



At PH 7.4 the forward reaction is essentially irreversible. Detection of elevated levels of S-Sulphanate compounds in an organ or tissue is evidence that it has recently been exposed to sulphite (*Gunnison and Palmes, 1978; Gunnison and Farruggella, 1979; Gunnison et al., 1981 b,c*).

MATERIAL AND METHODS

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1- Plant materials:

Seeds of *Vicia faba* (Var. Giza 2), *Hordeum Vulgare* (Var Giza 124) and bulbs of *Allium cepa* (var. Shandawil 1) were used in the present studies to investigate the cytogenetic effects of sodium sulphite solution.

2- Experimental agent:

Sodium sulphite solution was prepared by dissolving Na_2SO_3 in distilled water at room temperature ($23 \pm 1^\circ\text{C}$). One Mol of sodium sulphite solution was prepared by dissolving 126 gm in one liter.

Experiments were conducted to determine the lethal concentration of the experimental agent. Sodium sulphite solutions at the sublethal concentrations 0.01, 0.005, 0.0025, 0.00125, 0.00063, 0.00031, 0.00016 and 0.00008 Mol were used in the different experiments.

3- Effect on survival and early growth stages:

Seeds of *Vicia faba* (var. Giza 2) and *Hordeum Vulgare* (Var. Giza 124) were soaked in tap water for 24 hrs., then germinated in rolls of filter paper in glass cylinders with 2 cm. high distilled water at the bottom. After germination, the roots were immersed in the experimental agent for 6, 12 and 24 hrs. The seedlings were 4 days old at the end of treatments, after which, the number of survived plants was recorded and the length of



the shoot and the root was measured. The seedlings were washed thoroughly with tap water and continued their growth in rolls of filter paper. Readings of shoot and root-length were recorded after 3 and 6 days. Three replicates were conducted for each treatment and 15 seeds were used for each replicate.

In case of *Allium cepa* (var. Shandawil 1), bulbs of approximately equal size were chosen and germinated on the top of glass tubes filled with distilled water. Water was changed daily to obtain suitable aeration. When the roots reached a length of 2-3 cm., the roots were immersed in the experimental agent for different periods of time (6, 12 and 24 hrs.). The roots were 4 days old at the end of treatment, after which, the number of survived plants was recorded and the root-length was measured. The roots were washed with tap water and continued their growth in distilled water. Root-length was measured again, when they were 3 and 6 days old after treatment. Three replicates were conducted for each treatment and 9 bulbs were used for each replicate.

4- Effect on mitosis:

i- Treatment:

Seeds of *Vicia faba* (Var. Giza 2), *Hordeum vulgare* (Var. Giza 124) as well as bulbs of *Allium cepa* (var. Shandawil 1) were germinated. When the roots were treated with the experimental agent as previously mentioned. The roots were cut after treatment and fixed. Some of the roots treated for 24 hrs. with 0.07 and 0.0025 Mol were left in fresh water to recover for a period of 24 and 48 hrs.