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IMPACT OF AQUEOUS SULPHUR DIOXIDE ON BIOCHEMICAL PARAMETERS OF *RUMEX HESTATA*

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ABSTRACT

The impact of aqueous SO_2 on total chlorophyll, phaeophytin, carotenoids, proteins, amino acids, starch, and free sugars of a perennial herb, Rumex hestata was studied for a period of one year. All these, except free sugars, showed a remarkable decrease, attributed to the over all decrease in enzymatic activities, as compared to that under controlled conditions on exposure to sulphur dioxide. The increase in free sugars is attributed to the fact that hydrolytic enzymes get activated on SO_2 treatment and break polysaccharides.

INTRODUCTION

About 300 million tonnes of pollutants are being released annually into the atmosphere throughout the world. India alone contributes 10 million tonnes to it in the form of particulates and gaseous pollutants (Ganai *et al.*, 1996). In general, any fuel, which contains sulphur, produces SO_2 on combustion and almost all fuels contain some amount of sulphur, which is considered to be the most important phytotoxic molecule (Legge *et al.*, 1998). SO_2 toxicity to vegetation is well documented and its effects on vegetation have been well reviewed in terms of foliar injury (Malhotra and Khan, 1984; Pavgi *et al*. 1991; Venkateshwar *et al.* 1992), physiological and biochemical alterations (Agarwal, 2000 and 2003; Holopainen *et al.* 1993; Kailunainen *et al.* 1995) and species specific responses of plants to SO_2 (Jones *et al.* 1979; Farooq and Beig 1980;). Relations between genetic make up, concentration of the pollutants and prevailing ecological conditions have been studied by various workers like Heek *et al.* 1965; Ali 1993; Riga *et al*. 2005. SO_2 has also been seen effecting the metabolism and pigment destruction (Peiser and Yang, 1977; Wang *et al*, 2005). SO_2 gets well absorbed into the cells affecting various metabolic activities (Pahlich *et al.* 1972). SO_2 entering the gas phase of a leaf rapidly dissolves in the aqueous phase of cell wall (apoplastic space) and reacts with water to form bisulphite. Aqueous solution of SO_2 contains different species such as H_2SO_3, HSO_3^-, and SO_3^{2-} and dissolved but unreacted SO_2 (SO_2. H_2O) depending upon the pH of cell (Hocking and Hocking, 1977). The abnormalities caused due to SO_2 include: Membrane damage, plasmolysis, chlorophyll destruction and Genetic material destruction (Seema *et al.* 1991).

MATERIALS AND METHODS

(i) Preparation of aqueous sulphur dioxide

Aqueous sulphur dioxide of 1000ppm concentration

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was prepared by dissolving 1.8g of sodium sulphite in 500mL-distilled water. From this solution, the solutions of different concentrations like 250ppm, 500ppm and 750ppm were prepared by appropriate dilutions.

(ii) Treatment of leaf discs with aqueous sulphur dioxide

1g leaf discs of equal dimensions (1 cm dia.) of *Rumex hestata* (locally called as "ABUJ") were treated with different concentrations (250ppm, 500ppm 750ppm and l000ppm) of aqueous sulphur dioxide for 4hrs. in glass petri dishes under the light provided by a 100W tungsten electric bulb. According to the recommendations given by Environment Protection Agency (USA) for treating leaf discs with toxicants for primary screening of plants for their sensitivity. Each concentration was compared with the "control" that was run parallel with each sample.

The chlorophyll content was estimated according to the equation given by Strain *et al.* (1971), the phaeophytin according to Vernon (1960) and the carotenoid content was estimated following Duxbury and Yentech (1956). Proteins were estimated according to the standard methodology of Lowry *et al.* 1951 using 100mg% egg albumin as standard. Estimation of amino acids was carried out following the methodology of Lee and Takahashi (1966), that of starch was done according to Montgomery (1982).

RESULTS AND DISCUSSION

Table1. and 2. show the impacts upon chlorophyll, phaeophytin and carotenoid content of *Rumex hestata* on exposure to different concentrations of aqueous SO_2. Total chlorophyll content showed 66.9%, 70.9%, 75.2% and 81.2% decrease at 250 ppm, 500 ppm, 750 ppm and 1000-ppm SO_2 concentrations respectively. Such decrease could be attributed to overall decrease in enzymatic processes. The results are in conformation to the findings of Huang *et al.* (1994 a, b) and Vogelman and Borett (1988).

Similar decreasing trend was depicted by phaeophytin and carotenoids. Total phaeophytin content showed 62.3%, 66.1%, 67.2% and 73.4% decrease at 250 ppm, 500 ppm, 750 ppm and 1000 ppm SO_2 respectively, as compared to that of control. The respective decrease in carotenoids was recorded to

Table 1. Impact of different concentrations of aqueous sulphur dioxide on chlorophyll content

Parameter	Concentrations (ppm)				
	Control	250	500	750	1000
Chlorophyll 'a' (µg/mL)	1.13841±0.05	0.46917±0.03 (-58.7)	0.42536± 0.04 (-62.6)	0.35829 ± 0.10 (-68.5)	0.32404± 0.05 (-71.5)
Chlorophyll "b' (µg/mL)	2.35713±0.04 (-70.8)	0.68617± .006 (-74.9)	0.59141± .025 (-78.4)	0.50701± 0.028 (-85.9)	0.33181± 0.03
Total Chlorophyll (µg/mL)	3.49554 ± 0.08	1.15534± 0.04 (-66.9)	1.01677± 0.05 (-70.9)	0.8653 ± 0.054 (-75.2)	0.65585± 0.06 (-81.2)

Data represents the average of three samples analyzed separately ± S.D values in brackets represent %age decrease (-) compared to control.

Table 2. Impact of different concentrations of aqueous sulphur dioxide on phaeophytin and carotenoid content

Parameter	Concentrations (ppm)				
	Control	250	500	750	1000
Phaeophytin 'a' (µg/mL)	3.6713 ± 0.12	1.5937 ± 0.05 (-56.5)	1.4976 ± 0.06 (-59.2)	1.3590 ± 0.108 (-62.9)	1.1211 ± 0.06 (-69.4)
Phaeophytin 'b' (µg/mL)	5.8242 ± 0.41	1.983 ± 0.051 (-65.9)	1.7129 ± 0.06 (-70.5)	1.6082 ± 0.15 (-72.3)	1.3987± 0.06 (-75.9)
Total Phaeophytin (µg/mL)	9.4955 ± 0.18	3.5767 ± 0.06 (-62.3)	3.2105 ± 0.12 (-66.1)	3.0572 ± 0.12 (-67.8)	2.5198 ± 0.04 (-73.4)
Carotenoid (µg/ml)	0.2043 ± 0.058	0.1725 ± 0.06 (-15.5)	0.1683 ± 0.054 (-17.6)	0.1573 ± 0.05 (-23.0)	0.1425 ± 0.106 (-30.2)

Data represents the average of three samples analyzed separately ± S.D values in brackets represent or %age decrease (-) compared to control.

Table 3. Impact of different concentrations of sulphur dioxide on protein and amino acid content

Parameter	Concentrations (ppm)				
	Control	250	500	750	1000
Protein (mg/mL)	0.0105 ± 0.108	0.0093 ± 0.108 (-11.4)	0.0088±0.05 (-16.1)	0.0079±0.105 (-24.7)	0.005±0.106 (-52.3)
Amino acid (mg/mL)	0.0396±0.014	0.0319±0.010 (-19.4)	0.0281±0.010 (-29.0)	0.0269±0.006 (-32.0)	0.0247±0.004 (-37.6)

Data represents the average of three samples analyzed separately ± S.D. Values in brackets represent %age decrease (-) compared to control.

Table 4. Impact of different concentrations of aqueous sulphur dioxide on Free Sugars and starch content

Parameter	Concentrations (ppm)				
	Control	250	500	750	1000
Starch (mg/mL)	0.2503 ± 0.04	0.2351 ± 0.04 (-6.0)	0.2093 ± 0.02 (-16.3)	0.179 ± 0.01 (-28.4)	0.1508 ± 0.03 (-39.7)
Free Sugars (mg/mL)	0.129 ± 0.007	0.1494 ± 0.007 (+15.8)	0.1778 ± .008 (+37.8)	0.2286 ± 0.008 (+77.2)	0.238 ± 0.008 (+84.4)

Data represents the average of three samples analyzed separately ± S.D values in brackets represent %age increase (+) or %age decrease (-) compared to control.

be 15.5%, 17.6%, 23.0% and 30.2% at 250 ppm, 500 ppm, 750 ppm and 1000 ppm SO_2, comparison to the of control.

Table 3 shows the impact of aqueous SO_2 on protein and amino acid content of *Rumex hestata*. The protein content was observed to decrease by 11.2%, 16.1%, 24.7% and 52.3% at 250 ppm, 500 ppm, 750 ppm, and 1000 ppm SO_2 concentrations respectively when compared to control as has also been advocated by Godzik and Linskin (1974), Malhotra and Sarkar (1979), Prasad and Rao (1982) and Yang (1973). Higher concentrations of SO_2 possibly break enzymes and protein disulphide bonds into thiosulphonates and thiols this might be the reason for observed decrease in proteins in the present study. With regard to amino acid content of *Rumex hestata*, again a decrease of19.4%, 29.6%, 32.0% and 37.6% was observed at 250 ppm, 500 ppm 750 ppm, and 1000 ppm SO_2 respectively. Probable reasons for such a decline in amino acid could be the disturbance in their synthesis, as SO_2 affects whole N_2- metabolism of plants. Another cause of decline of amino acids might be the loss of ultra structural organization of the cells and destruction of riboromes, as was opined by Godzik and Linskin (1974), Mlodzsionowski and Bailobok (1977), Soikkeli and Tuovinnen (1979) and Yang (1973).

Starch content of *Rumex hestata* was observed to decrease by 6.0%, 16.3%, 28.4% and 39.7% on exposure to250 ppm, 500 ppm, 750 ppm, and 1000 ppm SO_2 concentrations respectively (Table 4).

As against chlorophyll, carotenoids, phaeophytin, protein, amino acid and starch, *Rumax hestata* showed a general increase in its free sugar content. An increase of 15.8%, 37.8%, 77.2% and 84.4% was observed in free sugars content of Rumex *hestata* at 250 ppm, 500 ppm, 750 ppm and 1000 ppm SO_2 respectively (Table 4). This increase could be attributed to the breakdown of polysaccharides by the action of hydrolytic enzymes that get activated on SO_2 treatment (Farooq and Beg, 1982).

REFERENCES

Agrawal, M., Singh, B., Rajput, M., Marshal, F. and Bell, J.N. 2003. Effect of air pollution on peri-urban agriculture: a case study. *Environ Pollut*. 126 (3): 323-329.

Agrawal, S.B., Innes, J.L. and Haron, A.H. 2000. Research on "air pollution impacts on Indian forests". *Air Pollution and The Forests of Developing and Rapidly Industrializing Regions*. 165-187.

Ali, A.E. 1993. Damage to plants due to industrial pollution and their use as bioindicators in Egypt. *Environmental Pollution*. 81 : 251-255.

Beig, M.U. and Farooq, M. 1988. SO_2 resistance of Indian trees II Experimental evaluation and metabolic profile. *Water, Air & Soil pollution*. 40 : 317-326.

Chan, K.Y., Li, C.K., Lai, C.K., Ng, S.F. and Chan, A. Y. 2003. Infantile isolated sulphite oxidase deficiency in a Chinese family : a rare neurodegenerative disorder. *Hong Kong Med. J.* 8 : 279-282.

Duxbury, A.C. and Yentech, C.S. 1956. Plankton pigment

monographs. *J. Mar Res.*15 : 19-101.

Farooq, M. and Beig, M.U. 1982. Effect of acute level of sulphur dioxide on barley seedlings. *Indian J Environ. Health* 24 : 215-21.

Farooq, M.and Beig, M.U. 1980. Effect of aqueous SO_2 on the membrane permeability of common Indian tree leaves. *New Botanist* 7 : 213-217.

Ganai, B.A. 2003. *Sulphite oxidase from Malva sylvestris.* Ph.D. Thesis, Deptt. of Biochemistry, Univ. of Kashmir.

Ganai, B.A., Masood, A., Amin, S., Iqbal, J. and Hamid, R. 1996. Effect of gaseous air pollutants on enzyme activities. *Oriental Science.* 83-87.

Godzik, S. and Linskens, H.F.1974. Concentration changes of free amino acids in pry bean leaves after continuous and interrupted sulphur dioxide fumigation and recovery. *Environmental Pollution.* 7 : 25-38.

Hao, J., Li, J., Duan, L., He, K. and Dai, W. 2002. Valuation of forest damage cost from SO_2 emission: a case study in Hunan Province, China. *HuanJing Ke Xue.* 23 (6) : 1-5.

Huang, Longhin, Murray, Frank, Yang and Xiaohong 1994 a. *Agric. Ecosyst. Environ.* 48 : 163-178.

Huang, Longbin, Murray, Frank, Yang and Xiaohong 1994 b. *Agric. Ecosyst. Environ.* 47 : 335-351.

Holopainen, T., Anttonen, S., Wulff, A., Palomaki, V. and Karenlanpi, L. 1992. Comparative effects of gaseous pollutants, acidic deposition and mineral deficiencies: structural changes in the cells of forest plants. *Agriculture*, *Ecosystem & Environ.*42 : 365-398.

Hocking, D. and Hocking, M.B. 1977. *Environ. Pollution.* 9 : 57-64.

Jones, H.C., Weatherfor, F.P., Noggle, J.C., Lee, N.T and Cunnigham, J.R. 1979. Power plant siting. Assessing of SO_2 effect on Agriculture. *Air Pollution Control Assoc. Pittsburgh, 72nd Annual Meeting Cincinnatti, USA. Reprint No.* 79-135.

Khan, A.A. and Malhotra, S.S. 1977. Effect of aqueous SO on Pine needle glycolipids. *Phytochemistry.*16 :539-543.

Kumar, S. and Prakash, C.B. 1977). Air pollution from SO_2 emission in India. *Chemical Age of India.* 28 : 465-472.

Longaves, R., Gomory, D., Paule, L., Blada, I., Popescu, F., Mankovska, B., Muller-Starck, G. Schbert, R., Percy, K., Szaro, R.C. and Karnosky, D.E. 2004. Genetic effects of air pollution on forest tree species of the Carpathian Mountains. *Environ Pollut.* 130 (1) : 85-92.

Legge, A.H., Jager, H.J. and Krupa, S.V. 1998. Sulphur dioxide. In: *Recognition of Air Pollution Injury to Vegetation: A Pictorial Atlas,* 2nd Ed.(ed. R.B. Flagler). pp.3-1-3-42. Air & Waste Management Asso. Pittsburgh.

Lee, Y.P. and Takahashi, T. 1966. An improved colorimetric determination of amino acids with the use of ninhydrin. *Anal.Biochem.*14 : 71-77.

Lowry, O.H., Rosebrough, N.J., Farr; A.L. and Randall, R.J. 1951. Protein measurement with Folin-phenol reagent. *J. Biol. Chem* 193 : 265-275.

Mlodzionowski, F. and Bialobok, S. 1977. The effect of sulphur dioxide on ultrastructural organization of larch needles. *Acta Societatis Botanicorum Po-loniae.* 46 : 629-634.

Montgomery, R. 1982. Determination of glycogen by Phenol-sulphuric acid method. *Arch. Bioch. Biophy.* 67 : 378-386

Noji, M., Saito, M., Nakamura, M., Aono, M., Saji, H. and Saito, K. 2001. Cysteine-synthase over expression in tobacco confers tolerance to sulphur containing environmental pollutants. *Plant Physiology.* 126 : 973-980.

Pavgi, S., Farooq, M., Venkateshwar, C. and Beg, M. U.(1991). Physiological and Biochemical effects of SO_2 on wheat varieties. *Environment & Ecology.* 9 : 760- 765.

Prasad, B.J. and Rao, D.N. 1982. Relative sensitivity *of* a leguminous and a cereal crop to sulphur dioxide pollution. *Environ. Pollution.* 29 (Ser.A) : 57-70.

Peiser, G.D. and Yang, S.F. 1977. Chlorophyll destruction by the bisulphite oxygen system. *Plant physiology.* 60 : 277-281.

Pahlich, E., Jager, H. and Steubling, L. 1972. Bee influssing der aktivakion von glutamate dehydregenase, glutamate synthetase,erberkeimligen.dural sulpherdioxide. *Angerwandte Botanik.* 48 : 183-197.

Riga-Karandinos, A.N. and Saitanis, C. 2005. Comparative assessment of ambient air quality in two typical coastal cities in Greece. *Chemosphere.* 59 (8) : 1125-36.

Ruan, A., Min, H., Meng, Z. and Lu, Z. 2003. Protective effects of sea-buckthorn seed oil on mouse injury induced by sulphur dioxide inhalation. *Inhal Toxiecol.* 15 (10) : 1053-10558.

Russel, C.E., Pittman, J., Darrall, N.M., Williams, L.E. and Hall, J.L. 1999). Effects of air pollutants on proton' and sucrose transport at the plasma membrane of *Ricinus communis. Plant, Cell and Environment.* 22 (2) : 221-227.

Seema, P., Farooq. M., Venkateshwar. C. and Beig, M.U. 1991. *Environ. and Ecology.* 9 (3) : 760-765.

Strain, H.H., Bengavin, T.C. and Walter, A.S. 1971. Analytical procedure for isolation, identification, estil11ation, investigation of chlorophyll. In: *Methods in Enzymology.* (A.S. Pietro.ed), New York, Academic Press, 23 : 452-476.

Saunders, P.J.W. 1966. The toxicity of sulphur dioxide to Diplocarpon rosaewolf causing black spot of roses. *Ann. Appl. Bioi.* 58 : 103-109.

Venkateshwar, C., Pavgi, S., Farooq, M. and Beig, M. U. 1992. Cuticular and anatomical changes in maize plant on exposure to SO_2. Proc. *Nat. Acad. Sci. India.* 62 (b) II : 512-517.

Vogelmann, F.A. and Borret, N. 1988. Effect of sulphur dioxide on photosynthetic pigments. *Environmental Experimental Botany.* 28 : 19- 25

Vang, S.F. 1973. Destruction of tryptophan during the aerobic oxidation of sulphite ions. *Environ. Res.* 6 : 395-402.

Vernon, L.P. 1960. Spectrophotometric determination of chlorophylls and phaeophytins in plant extracts. *AnalyJical Chern*. 32 : 1144-1150.

Wang, C., Xing, D., Zeng, L., Ding, C. and Chen, Q. 2005. Effect of artificial rain and SO_2 on characteristics of delayed light emission. *Luminescence*. 20 (1) : 51-56.

Wu, D. and Meng. Z. 2003. Effect of SO_2 inhalation on the glutathione redox system in mice and protective role of sea-buckthorn seed oil. *Arch Environ Contam Toxicol*. 45 (3) : 423-428.

Winner, W.E., Mooney, H.A. and Goldstein, R.A. 1985. Sulphur dioxide and vegetation. *Physiology*, *Ecology and Policy Issues*, Stanford Univ. Press, Stanford, California, pp. 96- J 17.

Zhang, I., Huang, S., Peng, S. and Vergara, B.S. 1994. Effect of elevated UV-B Radiation on abscisic acid and indole-cteic acid content of rice leaves. *Int. Rice Res. News*. 19 : 56-57.