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Science of the Total Environment 368 (2006) 627–636

Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Humankind

www.elsevier.com/locate/scitotenv

Biomarkers of oxidative stress in *Wallago attu* (Bl. and Sch.) during and after a fish-kill episode at Panipat, India

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Received 5 December 2005; received in revised form 18 April 2006; accepted 19 April 2006
Available online 5 June 2006

Abstract

The present investigation was carried out by sampling water, sediment and fish during a fish-kill episode at Panipat (Haryana, India), and again sampling at the same site was conducted after a gap of two months. During the second sampling no fish-kill was observed and the water was relatively less turbid and clear. Antioxidant profile and lipid peroxidation (LPO) in fish tissues were studied. Analysis was also carried out on the physico-chemical characteristics of water samples along with heavy metal and pesticide analysis in water and sediment samples during and after the episode. Dissolved oxygen level was substantially low during the fish-kill episode. Heavy metals (copper and chromium) and pesticides like BHC (Benzene hexachloride), DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) were also detected during the episode in water and sediment samples. Various oxidative stress biomarkers in liver, kidney and gill tissues in the Indian freshwater fish *Wallago attu* (Bl. and Sch.) collected from the site were investigated. The levels of reduced glutathione and non-protein thiol were significantly ($P<0.001$) higher in the liver of *Wallago attu* collected from Panipat after the fish-kill episode. Ascorbic acid levels in all the tissues did not change significantly after the episode. The LPO in liver, kidney and gills was significantly low ($P<0.01$–0.001) in all tissues of fish collected after the fish-kill episode. The protein carbonyl levels were significantly low ($P<0.05$–0.01) in all the fish organs sampled after the fish-kill episode. The findings suggest that industrial effluent may result in the massive loss of a commercial commodity. The simultaneous measurement of the physicochemical parameters of the water samples showed a good correlation between the biomarkers responses and the environmental chemical stress conditions.

Keywords: Aquatic pollution; River Yamuna; Fish-kill; Oxidative stress; Antioxidants; Lipid peroxidation; Protein oxidation

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0048-9697/$ - see front matter
doi:10.1016/j.scitotenv.2006.04.011

1. Introduction

Acute effects such as fish-kills and water discoloration occurred in the 1800s in the USA (e.g. New York and Boston Harbors) and the UK (e.g. the river Thames). Chlorinated hydrocarbon insecticides were strongly implicated in having caused the large fish-kill by changing the number of leucocytes in channel catfish (Zeeman and Brandley, 1981) on the Mississippi River. Analysis of blood collected from dying fish showed the low white blood cells count. Gill et al. (1988) have reported the effect of aldicarb poisoning in the peripheral blood of fish (*Puntius conchonius* Hamilton). Microbiological infections have also been implicated in some large scale fish-kill episodes (Zeeman and Brandley, 1981; Munoz et al., 1994). A wide variety of both inorganic and organic pollutants are present in effluents of breweries, tanneries, dyeing and textile units, paper and pulp mills, steel industries, mining operations etc. The pollutants include oils, greases, plastics, plasticizers, metallic wastes, suspended solids, phenols, toxins, acids, salts, dyes, cyanides, pesticides etc. Many of these pollutants are not easily susceptible to degradation and thus cause serious pollution problems. Contamination of ground water and fish-kill episodes are the major effects of the toxic discharges from industries (Chandra, 1985; Chawla et al., 1986).

Environmental monitoring by means of biomarker parameters assessed in different species is a useful tool. It has the advantage of providing a quantitative assessment as well as valuable information of ecological relevance on the adverse effects caused by water pollution. Measurement of physical, chemical and a few biological characteristics has been the yardstick for the traditional assessment of river quality. With a view to identifying the structural or functional integrity of ecosystems, measurements of aquatic biota have gained considerable interest in the assessment of river condition in the recent past. This process is often seen as analogous with human health. In order to proceed from revealing of contamination, which is a physico-chemical phenomenon, to an assessment of pollution, which implies a demonstration of effects on biota, biological techniques must be applied preferably in concert with chemical measurements (Bayne et al., 1988). Dissolved oxygen (DO) is one of the more important parameters available in the field of water pollution control, as it permits the evaluation of the aerobic conditions of a water-course, which receives discharge of pollutants. Thus, measurement of the DO concentration in water allows the prediction and study of the phenomena of photosynthesis and corrosion action. Biomarkers are being extensively used for the assessment of the health of river biotic community in several countries. It has also been suggested that oxidative stress biomarkers could be employed in environmental monitoring programs (McCarthy and Shugart, 1990). Over the past few years, increasing emphasis has been placed on the use of biomarkers as tools for monitoring both the environmental quality and the fitness of organisms inhabiting ecosystems (Stegeman et al., 1992). The potential usefulness of oxyradical-mediated responses to provide biochemical markers for biomonitoring gave rise to studies on antioxidant defenses (DiGiulio et al., 1989; Winston and DiGiulio, 1991). Non-enzymatic antioxidants have been successfully employed in aquatic biomonitoring studies (Hasspieler et al., 1994; Pandey et al., 2003). Use of protein carbonyls as biomarkers of exposure in fish has also been explored recently (Almroth et al., 2005; Parvez and Raisuddin, 2005).

The Yamuna River is one of the heavily polluted rivers in India. Pollutants flowing into the river are contributed from the waste of the cities situated along its bank (Pandey et al., 2003; Aleem and Malik, 2005). During the survey and sample collection at Panipat, in the month of August 2001, a large fish-kill episode was recorded. Surviving fish along with the water samples were also collected from the site. After a gap of two months another sampling was made in October, 2001. During that sampling no fish kill was observed and water was also clear. One species common in both the samplings was *Wallago attu* (Bl. and Sch.). Previously we have reported the enzymatic antioxidants as biomarkers of this fish in a field study of Yamuna River (Pandey et al., 2003). In the present study we wanted to study the potential of using non-enzymatic antioxidants as biomarkers of aquatic pollution and make a comparison of biomarker responses of the fish collected during the fish kill episode and after the episode. Antioxidant profile and lipid peroxidation (LPO) in fish tissues were studied. Physicochemical characteristics of water samples along with the heavy metal and pesticide content in water and sediment samples were also analysed for both the samplings.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA), 2,4-dintrophenylhydrazine (DNPH), guanidine hydrochloride, ethylene diamine tetraacetic acid (EDTA) disodium salt, sulphosalicylic acid, 5,5′ dithio-bis (2-nitrobenzoic acid) and DTNB were purchased from Sigma-Aldrich Co., St. Louis, MO, USA. Other routine chemicals and reagents, obtained from local sources, were of high quality and purity.