Environmental Nanotechnology, Monitoring & Management 4 (2015) 74–84

Contents lists available at ScienceDirect

Environmental Nanotechnology, Monitoring & Management

journal homepage: www.elsevier.com/locate/enmm

Phytotoxicity of River Chenab sediments: In vitro morphological and biochemical response of *Brassica napus* L.

Attarad Ali[a,c], Abdul Rehman Phull[b], Muhammad Zia[c,*], Ali Musstjab Akber Shah[b], Riffat Naseem Malik[a], Ihsan ul-Haq[d]

[a] Department of Environmental science, Quaid-i-Azam University, Islamabad 45320, Pakistan
[b] Department of Biochemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan
[c] Department of Biotechnology, Quaid-i-Azam University, Islamabad 45320, Pakistan
[d] Department of Pharmacy, Quaid-i-Azam University, Islamabad 45320, Pakistan

ARTICLE INFO

Article history:
Received 17 March 2015
Received in revised form 2 July 2015
Accepted 13 September 2015

Keywords:
Antioxidant
Enzymatic activities
River Chenab
Sediment
Phytotoxicity

ABSTRACT

River Chenab is one of the major sources of water for irrigation of agriculture fields in Punjab, Pakistan. The present investigation was conducted to appraise the effect of river Chenab sediments on growth; morphological and biochemical prospects of in vitro grown *Brassica napus* seedling. A total 19 residue samples collected from different sites of River Chenab were evaluated. The sediments extracts, in most of the cases, significantly influenced on final germination, rate of germination and mean period of final germination of *B. napus* seedlings in comparison with controls. Reduction in root length was observed as compared with shoot length. Decrease in relative dry weight of seedlings ranging from 45% to 64% was also examined. Biochemical analysis revealed that the sediments extracts tend to increase in total protein and total phenolic contents in *B. napus* plants while variation in MDA and flavonoid contents were observed as compared with control. Increase in chlorophyll a & b and carotenoid contents were also observed in plants germinated in the presence of sediments extracts except sample 4. The enzymes (POD, SOD and protease); responsible to mitigate hazardous effects of sediment contamination; were found elevated in the seedlings. Phytotoxic evaluations of sediments demonstrate that it is consistent and practical tool for assessing quality of sediment. However, increased activities of antioxidants; enzymes and proteins favor the adaptation or tolerance to contamination by the seedlings.

1. Introduction

The River Chenab is one of the most important rivers in Pakistan that flows in from India at the upstream of rim station Marala, which has the total catchment of almost 38,000 Km^2. In Punjab, this river transverse 560 km through highly contaminated and industrial cities like Faisalabad, Gujranwala, Sialkot, Gujarat, Jhang, Khanewal and Multan. It is a major source of irrigation that fulfills the domestic, agricultural, and industrial water necessities of these regions (Bhatti and Latif, 2011). Several factors like reduction in water flow, industrial effluents, pesticides and fertilizers being added from surrounding fields are exhaustively degrading the water quality of Chenab River. Eqani et al. (2012a) have reported the persistent organic pollutants, poly-chlorinated biphenyls, organochlorine pesticides, and polycyclic-aromatic hydrocarbons in the water of Chenab River. The river catchment area is also contaminated by plenty of dichloro diphenyl trichloroethane (DDTs) discharging from pesticides manufacturing factories (Eqani et al., 2012b; Malik and Nadeem, 2011).

Sediments are the particles of different composition, size and from that deposit at the bottom of aquatic environment (Hudson-Edwards et al., 2003). They act as natural sponges that adsorb pollutants present in water (Malik and Nadeem, 2011); detoxicate the flowing water and also serve as foot prints of the quality of water passing there by. These contaminated sediments not only affect aquatic life (Ingersoll et al., 2002) but also alter the physiology and morphology of plants both at cellular and organ levels (Bornette and Puijalon, 2011; Wang et al., 2004). The pollutants also interfere with enzymes involved in seed germination, growth and protein synthesis (Dupuy et al., 2015).

Abbreviations: FG, final germination; MPFG, mean period of final germination; MDA, malondialdehyde; POD, peroxidases; RL, root length; ROS, reactive oxygen species; RG, rate of germination; RDW, relative dry weight; RWC, relative water content; SL, shoots length; iSG, seedling growth; SOD, superoxide dismutases; WC, water content.

* Corresponding author.
E-mail address: ziachaudhary@gmail.com (M. Zia).

http://dx.doi.org/10.1016/j.enmm.2015.09.003

The compounds present in seed/plant vicinity may affect cellular permeability (Rouhier et al., 2008), physiology, electron transport in photosynthesis (Liu et al., 2009), variation in enzymatic activities (Dupuy et al., 2015), and growth inhibition (Wang et al., 2004). However, plants have systems to tolerate stresses through biochemical and enzymatic approaches. The plants have some interaction between antioxidant system and resistance to environmental stresses (Bor et al., 2003). The stress increases the generation of reactive oxygen species (ROS) within the cells which may result in peroxidation of membrane lipids, damage nucleic acids, amino acids and proteins (Stoeva and Bineva, 2003) that may cause cellular injury, metabolic changes and cell death. Plants have ROS scavenging mechanism through phytochemicals such as free radical scavinging molecules and enzymes i.e. peroxidases (POD), superoxide dismutases (SOD) etc. to prevent the oxidative damage (Apel and Hirt, 2004; Foyer and Noctor, 2005).

Model plant or standard target specie in scientific research must have ideal characteristics i.e. high rate of seed germination and rapid growth rate of seedling. Various phytotoxicity assays have been standardized by such model plants (Macías et al., 2000). In this study *Brassica napus* L. was selected as a target plant. It has all the ideal scientific features to be chosen as model dicotyledonous representative (Giavalisco et al., 2006). In current study *B. napus* L. seed germination potential, seedlings length and weight loss and biochemical prospects such as molecular and enzymatic antioxidative agents were used as bioindicator for the evaluation of sediment toxicity of River Chenab Punjab Pakistan.

2. Materials and methods

2.1. Study area

The river Chenab stretches from headwork Marala to Punjnad headwork in the Punjab province Pakistan. A number of major cities exist at riverside famous for production of cash crops such as rice, wheat, sugarcane, cotton, mango, citrus etc. in agriculture lands and many industries are also established in these cities. There are 15 main points, from where pollutants and sewage are discharged in the river stream every day (Fig. 1). Among these, four are in Gujrat, three in Multan, two each in Mandi Bahauddin, Jhang, Chinot, one each in Hafizabad and Sargodha districts.

2.2. Sampling and Extraction of sediments

Surface sediments of various selected sites were collected from river stretch of about 500 Km starting from Marala to Punjnad station from May 2007 to November 2009. Locations of all sites were marked by using Global Positioning System (GPS-Garmin). The sites were selected on the bases of anthropogenic activities. All sampling sites are shown in the map (Fig. 1) and details of sites are given in Table 1. Sample in triplicate from each site was collected at 15–30 cm deep and within the distance of hundred meters. A total of 19 samples from different sites were taken in sterilized and labelled glass containers. Then samples were transferred to laboratory and stored in refrigerator at −20 °C.

The protocol described by Turker and Camper (2002) was followed after slight modification. In brief, Two gram of sediment was suspended in 16 ml sterilized distilled water and sonicated (E 30H Elmasonic) for 60 min with continuous shaking. The suspension was filtered and the filtrate was used to analyze growth modulating effect on *B. napus*.

2.3. Bioassay procedure

Bioassay was performed to assess the effects of sediments extracts on seed germination, seedling growth, seedling weight and water content of *B. napus*. In a Petri dish laid by double layer of Whatman filter paper No.1; 5 ml of the sediment filtrate with final concentrations equal to 125 mg/ml of sediment was added. Distilled water was used a negative control and 2, 4-D (500 and 1000 ppm) was used as a positive control (Pereda-Miranda et al., 1993). Under aseptic conditions, *B. napus* seeds were disinfected with aqueous solution of sodium hypochlorite (10%) for 5 min followed by thoroughly rinsed with autoclaved distilled water. A total

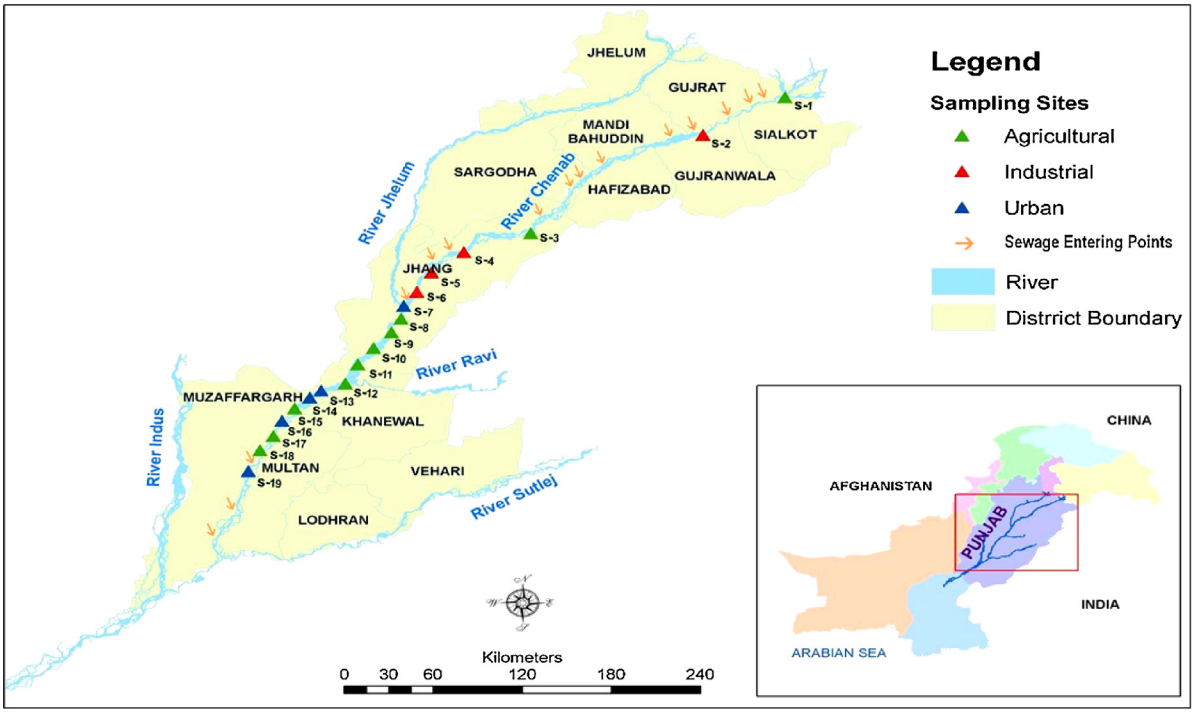

Fig. 1. Map of study area showing sampling locations of River Chenab, Punjab province Pakistan.