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

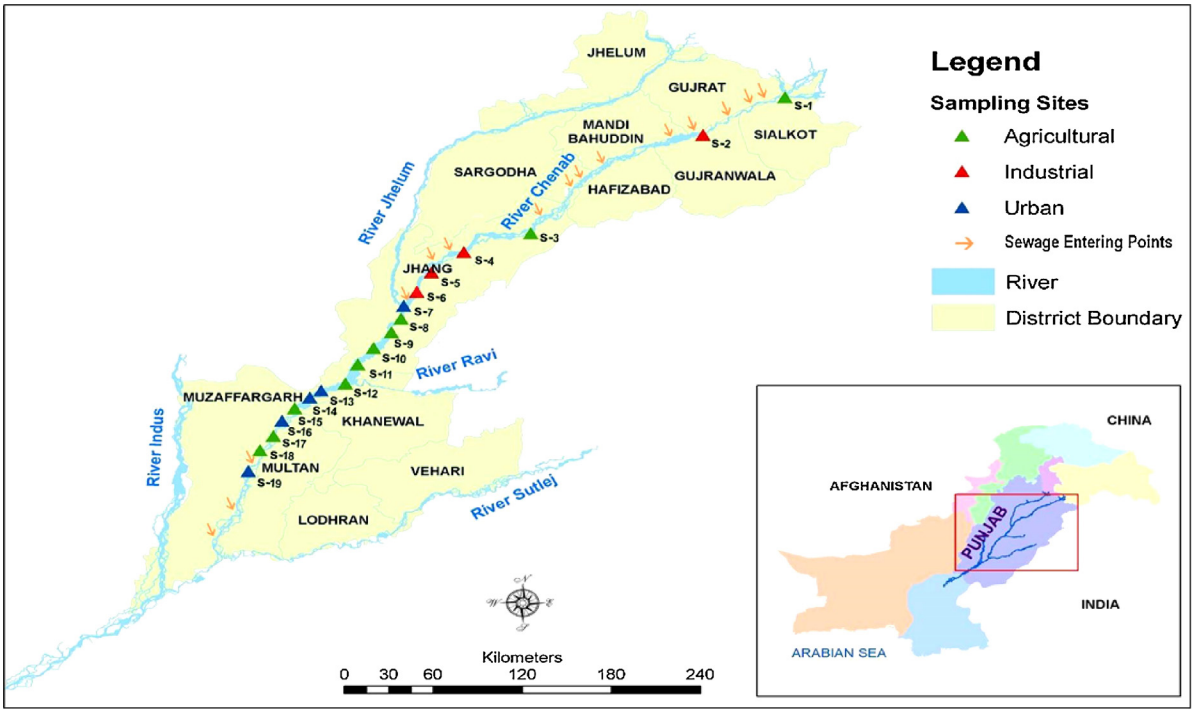

Fig. 1. Map of study area showing sampling locations of River Chenab, Punjab province Pakistan.

Table 1
Detailed description and location of the sampling sites.

Site	Lat.-Longitude	Site description
1	32°67′09.9″ N, 74°47′09.2″ E	Upstream of Marala rim station near at the Sialkot city where Chenab enters Pakistan.
2	32°40′29.2″ N, 73°97′25.2″ E	Sub-urban and agricultural area at the upstream of Chenab near Gujranwala district in Khanki Barrage.
3	31°70′61″ N, 72°93′53″ E	Agricultural area at the mainstream of Chenab in Chinyot district.
4	31°56′45″ N, 72°52′52″ E	Industrial drainage area receiving huge amount of pollutants, located in district Jhang.
5	31°54′43.3″ N, 72°33′12.3″ E	Urban area at the mainstream of Chenab, An industrial drain from Faisalabad city joins this site in district Jhang.
6	31°25′81.2″ N, 72°20′71.8″ E	Urban area at upstream of Chenab, 10 km above Trimmun Headwork in Jhang city.
7	31°18′7.5″ N, 72°16′46″ E	Sub-urban area at downstream of Trimmun headworks where river Chenab joins river Jhelum.
8	31°08′27.5″ N, 72°14′25.3″ E	Agricultural area, 10 km below Trimmun Headwork in Nikokara town.
9	30°99′20.7″ N, 72°08′65.3″ E	Agricultural area of the district Jhang.
10	30° 87′90.3″ N, 71°97′70″ E	Agricultural area of the district Jhang.
11	30° 76′19.3″ N, 71°88′23.1″ E	Agricultural area at upstream on the boundary region of district Jhang and Khanewal.
12	30°62′61.3″ N, 71°80′76.6″ E	Agricultural area at downstream of Chenab, 10 km below the joining point between river Ravi and Chenab.
13	30°57′63.3″ N, 71°65′99.7″ E	Sub-urban and agricultural area at upstream of Chenab in district Khanewal.
14	30°52′61″ N, 71°59′50.1″ E	Agricultural area of district Khanewal.
15	30°45′01″ N, 71°50′70.1″ E	Agricultural region of district Multan.
16	30°35′91.8″ N, 71°42′32.1″ E	Sub-urban and agricultural area at the joining point between Chenab and Indus link canal situated in Muzaffarghar district.
17	30°44′33″ N, 71°50′68″ E	Sub-urban area at the mainstream of river Chenab.
18	30°152′06″ N, 71°29′03.1″ E	Sub-urban area of district Multan.
19	30°07′48.2″ N, 71°28′42.7″ E	Urban area near at Shershah Bridge located in district Multan.

of 20 seeds having more than 98% germination were plated in each plate and the plates were kept in dark at 25 ± 1 °C. When emergent radical reached up to 2 mm, the seeds were considered as germinated.

Final germination percentage (FG%) is the maximum average percentage of germinated seeds;

$$\text{FG\%} = \left(\frac{\text{No of germinated seeds}}{\text{No of total planted seeds}}\right) \times 100$$

Rate of Germination (RG) is the time course of seed germination, number of seed germinated per day;

$$\text{RG} = \sum \frac{N_i}{D_i}$$

where N_i = daily increase in seedling number, D_i = number of days from seed placement.

Mean period of final germination (MPFG) is time related to daily increase in germination comparative to maximum germination;

$$\text{MPFG} = \Sigma \frac{N_i \times D_i}{S}$$

where S = Total number of seeds germinated.

2.4. Seedling growth, weight loss and water content analysis

Seedling growth, root length (RL), shoots length (SL) and seedling weight were measured at fifth day after sowing. From hypocotyl to shoot tip and root tip were measured as shoot length and root length, respectively. Digital balance was used to measure seedling fresh weight and the dry weight after drying at 70 °C in oven for 24 h.

Percent inhibition of seedling growth (% iSG) was calculated by the given formula;

$$\%\text{iSG} = \left[\frac{(\text{N} - \text{S})}{\text{N}}\right] \times 100$$

where

N = RL + SL of control or negative control, RL = root length, SL = shoot length.

S = RL + SL of sediment samples treated plants.

The percent inhibition of shoot and root length was calculated as

$$\text{Soot length inhibition(SLI\%)} = \left(\frac{\text{SL of control} - \text{SL of sample}}{\text{SL of control}}\right) \times 100$$

$$\text{Root length inhibition(RLI\%)} = \left(\frac{\text{RL of control} - \text{RL of sample}}{\text{RL of control}}\right) \times 100$$

Seedling growth is the emergence of the plantlet from soil. It can be measured by different ways like number of seedlings per unit area, time required for the emergence of plantlet top of seedlings mass and seedlings height as described by (Kandil et al., 2012).

Percentage seedling length (%SdL) was calculated using given formula:-

$$\%\text{SdL} = \left[\frac{\text{RLn} + \text{SLn} - \text{RLs} + \text{SLs}}{\text{RLn} + \text{SLn}}\right] \times 100$$

Percentage of weight loss (%WL) in seedling was found according to given formula:-

$$\%\text{WL} = \left[\frac{(\text{Wn} - \text{Ws})}{\text{Wn}}\right] \times 100$$

Percentage relative dry weight of seedlings was measured by given formula:-

$$\text{RDW\%} = \left[\frac{\text{DWs}}{\text{DWn}}\right] \times 100$$

Where n = Negative control, s = Sample.

Seedling fresh leaves weighing 200 mg were collected to determine water content and relative water content. Leaves were placed in distilled water and removed after 12 h and dried on tissue paper. Weights of turgid leaves were taken followed by oven drying in oven at 70 °C for 24 h. Equation as described by Turner (1986) was used to calculate percent of relative water content (RWC).

$$\text{WC}(\%) = \left(\frac{\text{FW} - \text{DW}}{\text{FW}}\right) \times 100$$

$$\text{RWC}(\%) = \left[\frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}}\right] \times 100$$

where RWC = relative water content, FW = fresh weight, DW = dry weight, TW = turgid weight.

2.5. Phytochemical evaluation of plants

Total chlorophyll and carotenoids content Chlorophyll a, b, a:b and total chlorophyll were measured as described by Ullah et al. (2013). In short, 100 mg leaves were grinded in 1 ml DMSO and

left for 24 h. Thereafter, the mixture was centrifuged at 15000 g for 30 min. Absorbance of extract was recorded at 645 nm and 663 nm for the estimation of chlorophyll a, b contents using spectrophotometer Chlorophyll a, b were calculated by the Formula and content was expressed as mg/g fresh weight of leaves.

$$\text{Chl a(mg/g FW of leaf)} = [0.0127(\text{OD663}) - 0.00269(\text{OD645})] \times \frac{v}{a} \times 1000 \times w$$

$$\text{Chl b(mg/g FW of leaf)} = [0.0229(\text{OD645}) - 0.00468(\text{OD663})] \times \frac{v}{a} \times 1000 \times w$$

where v = extract volume (5 ml); a = length of light path in cell (usually 1 cm); w = fresh weight of leaves in gram.

$$\text{Total chlorophyll content} = [0.0202(\text{OD645}) + 0.00802(\text{OD663})] \times 100$$

$$\text{Chl a ratio b} = \frac{\text{Chl a}}{\text{Chl b}}$$

Absorbance of extract was also checked at 480 nm and total carotenoids (mg/g FW) were calculated by using given formula

$$\text{Carotenoids} = \frac{\text{absorbance at 480} \times \text{extract volume}}{\text{fresh weight of leaves in grams}}$$

2.5.1. Malondialdehyde (MDA) content

Slight modified thio-barbituric acid (TBA) test as described by (Bailly et al., 1996) was followed for determination of malondialdehyde content. Absorbance of supernatant was observed at 532 nm (specific) and 600 nm (nonspecific). Nonspecific was deducted from specific absorbance value. Malondialdehyde (MDA) content as marker of lipid peroxidation was stated with extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as μmol/g FW.

2.5.2. Total phenolics determination

Folin-Ciocalteu reagent method as describe by (Haq et al., 2012) was followed for determination of total phenolic content. A total of 100 mg dried leaves were suspended in 1 ml methanol. An aliquot of 40 μl was mixed with 750 μl of ten time diluted Folin-Ciocalteu reagent. After 5 min, 750 μl of 6% Na_2CO_3 was added. The mixture was incubated for 90 min at room temperature and absorbance was recorded at 725 nm using spectrophotometer. Gallic acid was used as standard and a linear correlation at different concentrations (0.0–25.0 μg/ml) was drawn to find regression coefficient as ($y = 0.0732 \times -0.0205$ and $R^2 = 0.991$).

2.5.3. Total flavonoids content

Aluminum chloride colorimetric method as described by (Chang et al., 2002) was used for quantification of flavonoids content. Plant extract at 100 mg/ml in methanol was prepared. An aliquot of 25 μl was reacted with 100 μl of 10% Al_2Cl_3; 1.975 ml methanol and 100 μl of 1 M potassium acetate, 2.8 ml distilled water as was added and the reaction mixture was incubated at room temperature for 30 min. Thereafter absorbance was recorded at 415 nm using spectrophotometer. Flavonoids content was expressed in terms of quercetin equivalent. A linear correlation at different concentrations (0.0–25.0 μg/ml) was drawn to find regression coefficient as ($y = 0.0101 \times -0.004$ and $R^2 = 0.991$).

2.5.4. Total protein content (TPC)

Total protein extract was prepared as described by Nayyar and Gupta (2006) with slightly modifications.. Extraction buffer (50 mM potassium phosphate buffer with 1% PVPP at pH 7) was used to homogenize the seeds and centrifuged at 15,000 g at 4 °C for 30 min. The supernatant was collected and used for TPC analysis. Protein content was quantified using the Lowry method (Lowry et al., 1951) using Bovine serum albumin equivalents (BSA) as standard. Absorbance of reaction mixture was measured as 650 nm using micro-plate spectrophotometer (Biotech Elx-800).

2.6. Enzymatic activities

To study enzyme activity, 1 g plant material was ground in prechilled pestle and mortar in 2.5 ml extraction medium. The extraction medium comprised of 0.1 M phosphate buffer (pH 7.0), 10 mM KCl, 1.0 mM $MgCl_2$ and 10 mM EDTA. The ground material was centrifuged at 10,000 g for 20 min at 4 °C. The supernatant was used for enzyme assays.

2.6.1. Protease activity assay

Method as described by (Long et al., 1995) with minor modification was followed for the measurement of proteases activity. Casein was used as substrate and absorbance was measured at 660 nm. Activity was described as increase 0.l in absorbance of reaction mixture per unit time. Activity was expressed as units per gram of fresh weight.

Protease Activity = (OD/0.1) U × total volume of extract/g FW

2.6.2. Peroxidases (POD) activity assay

POD activity was determined by following the method of (Lagrimini, 1991) with some modifications. The assay mixture contained 200 μl of K-phosphate buffer, 100 μl of 100 mM Guaiacol, 100 μl of enzyme extract, 500 μl of distilled water, 100 μl of 27.5 mM H_2O_2. Reaction mixture was subjected to 10 readings for the gap of 20 s at 470 nm. Enzyme activity was then quantified using extinction coefficient of 6.39 mM^{-1} cm^{-1} and expressed as nmol/min/mg FW.

2.6.3. Superoxide dismutases (SOD) activity assay

Slight modified method as described by (Ullah et al., 2013) was followed for SOD activity. Briefly reaction mixture comprises on 100 μl of 1 mM EDTA (ethylene diamine tetra acetic acid), 100 μl of 0.75 mM NBT (nitro blue tetrazolium), 390 μl of 0.05 M phosphate buffer, 10 μl of 0.02 mM Riboflavin and 300 μl of extract. It was subjected to incubate for 7 min under florescent light. Absorbance of mixture was recorded at 560 nm. Activity of SOD was expressed as nmol/min/g FW using the reaction coefficient of 6.39 mM^{-1} cm^{-1}.

2.7. Statistical analysis

All the experiments were performed in triplicate; each replicate was a pool of 20 seeds. The results are presented as mean with standard deviation. Descriptive statistics, bivariate Pearson correlations and significance analysis ($p < 0.05$) were performed using the SPSS 11.0 (SPSS Inc.). Differences among treatment means were separated by the least significance difference (LSD) test at 0.05 probability level.

3. Results

3.1. Germination, weight and water content of B. napus plants

Germinating potential (final germination percentage (%FG), Rate of germination (RG), mean period of final germination (MPFG)) of *B. napus* seeds were affected by river sediment exposures. In

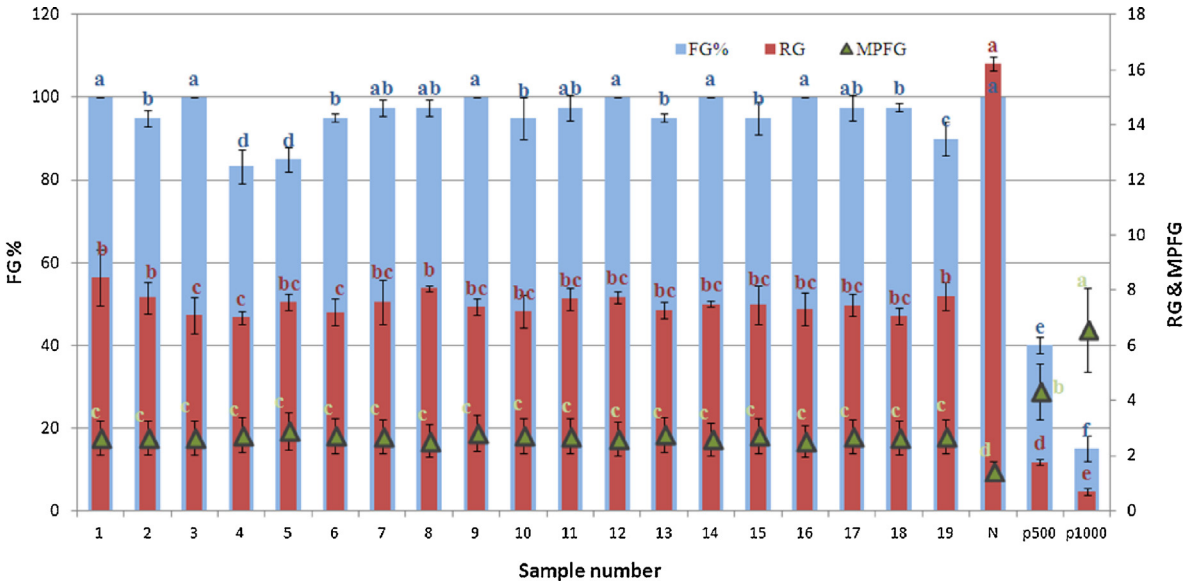

Fig. 2. Final germination (FG%), Rate of germination (RG) and mean period of final germination (MPFG) of *Brassica napus* plants in presence of River Chenab sediments extracts. The data expressed as mean with standard deviation ($n = 3$). Different small letters marked at data points are significantly different at the 0.05 probability level using LSD test. 1–19 samples referred as Table 1; N, negative control; p500 and p1000: 2,4-D 500 ppm and 1000 ppm, respectively.

most of the cases significant ($p < 0.05$) effect on final germination was observed as compared with controls. Final germination was ranged from 83% to 100% (Fig. 2). Lowest germination (83 and 85%) was recorded under sample 4 and 5 treatment, respectively. While sample 1, 3, 9, 12, 14, and 16 did not show any effect of final germination of *B. napus* seeds.. All the samples exhibited low rate of germination (RG) ranges from 7.01 to 8.45 significantly different at $p < 0.05$ in comparison with control. However, the difference was low among the treatments. The *B. napus* seeds treated with 2,4 dichloro-acetic acid at concentration of 500 and 1000 ppm showed 1.77 and 0.69 germination rate, respectively while negative control, water showed 16.22 rate of germination. A non-significant MPFG was also observed against all samples excluding controls. The maximum MPFG value of 2.89 was shown by the seedlings of sample 5 while the minimum was measured against sample 16 as 2.53 (Fig. 2).

The effect of sediments extracts on root and shoot length was examined on daily basis since the day of seeds placement up to the end of 5th day. A significant inhibition at $p < 0.05$ in root length, shoot length and seedling length were observed by all samples in comparison with negative control (Fig. 3). Sample 2, 4, 5 and 6 were found with highest root inhibitory effect among all samples (86.3–87.7%) while lowest by sample 1 and 4 (68.45 and 70.3%, respectively in comparison with control). All the 19 treatments may have inhibitory effect on shoot length of the tested plant. Seedlings of sample 6 treated exhibited 81.2% inhibition while sample 8 exhibited least inhibition (68.15%) (Fig. 3). Among all samples; samples 2, 6, 13 and 19 showed >80% inhibitions of seedling length

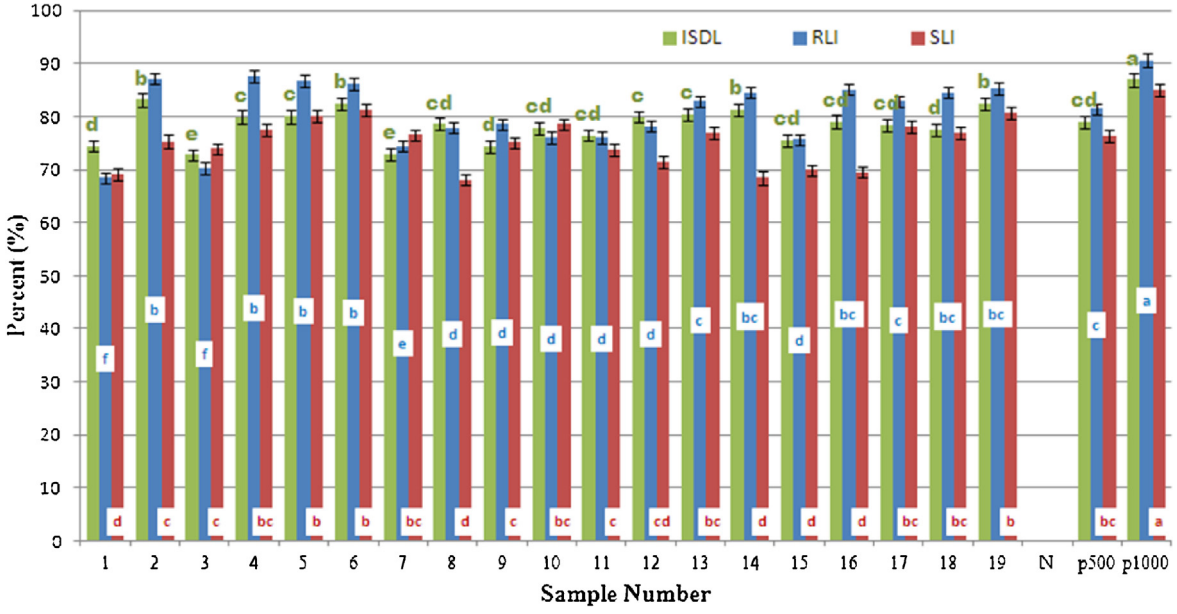

Fig. 3. Percent inhibition in the seedling length (ISDL), root length inhibition (RLI) and shoot length inhibition (SLI) of *Brassica napus* plants in presence of River Chenab sediments extracts. The data expressed as mean with standard deviation ($n = 3$). Different small letters marked at data points are significantly different at the 0.05 probability level using LSD test. 1–19 samples referred as Table 1; N, negative control; p500 and p1000: 2,4-D 500 ppm and 1000 ppm, respectively.

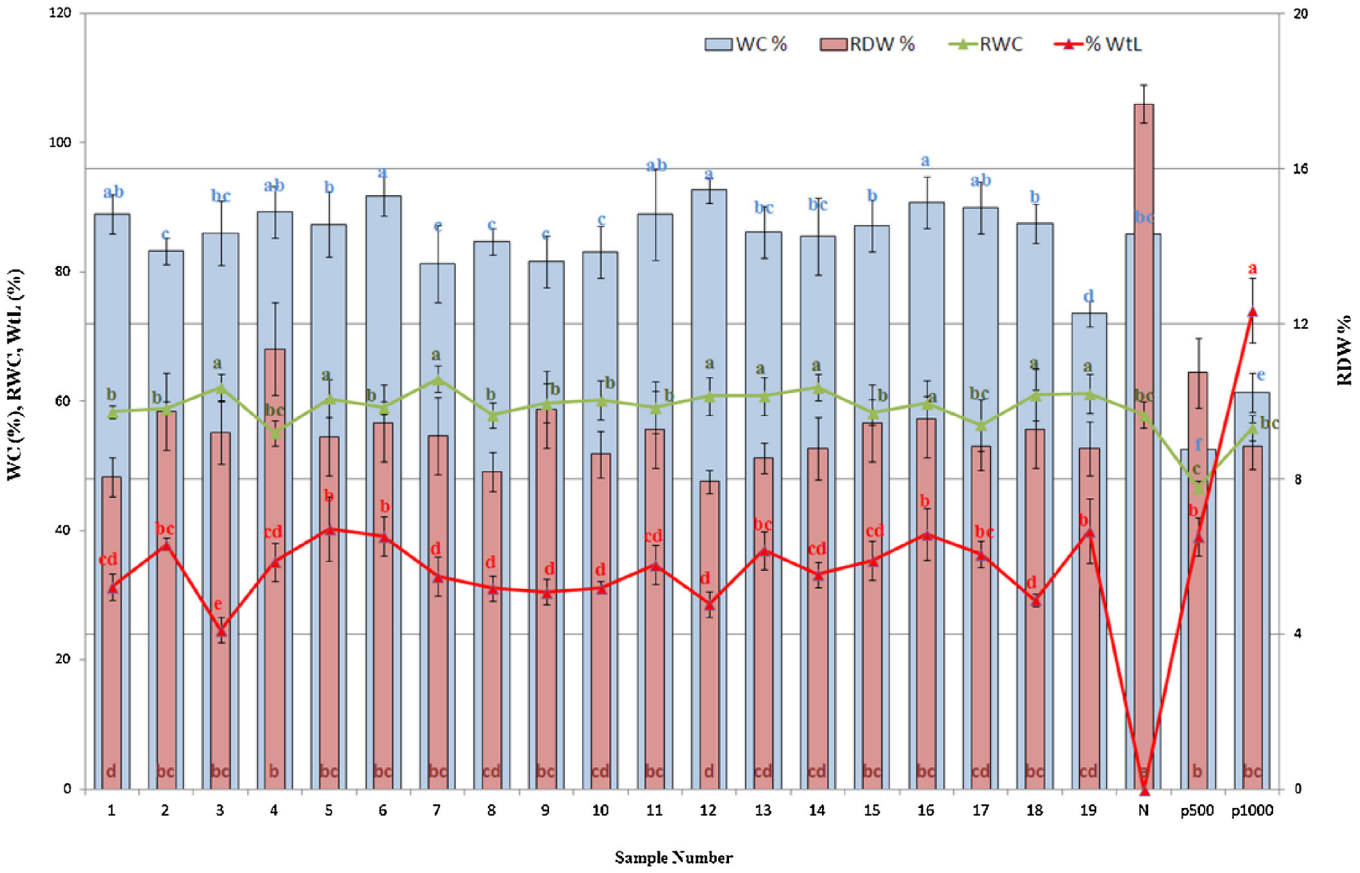

Fig. 4. Water content percentage (% WC), Relative dry weight percentage (RDW%) Relative water content (RWC) and Percent weight loss (% WtL) of *Brassica napus* plants in presence of River Chenab sediments extracts. The data expressed as mean with standard deviation ($n = 3$). Different small letters marked at data points are significantly different at the 0.05 probability level using LSD test. 1–19 samples referred as Table 1; N, negative control; p500 and p1000: 2,4-D 500 ppm and 1000 ppm, respectively.

(Fig. 3). The positive control 1000 ppm 2,4-D showed 87% inhibition. Samples 3 and 7 were found least inhibitory to seedling length.

All the 19 sediments samples caused significant decrease in weight of seedlings of target plant (Fig. 4). Seedlings of sample 5, 6 and 19 exhibited maximum weight loss (~40%) in comparison with negative control. While the sample 3 treatment exhibited 24% weight loss (Fig. 4). All the sediments samples also affected the RDW of seedlings. Lowest decrease in RDW of seedlings grown in sample 4 as 11.35% (64.23% less than control) and highest was recorded against sample 1 as 8.04% (45% less than control). Elevated water content was observed in seedlings of 13 samples, while 6 samples showed decreased in water content (Fig. 4). The seedlings of sample 6, 12 and 16 exhibited the maximum water content of 90–92% (Fig. 4). While seedlings of sample 19 showed minimum water content of 73.55% comparative to the control. *B. napus* seeds treated with 2,4 dichloro-acetic acid at 500 and 1000 ppm showed 55.1% and 61.3%, respectively. A non significant difference was observed in case of relative water content among the treatments. The seedlings of 16 samples showed higher RWC with a maximum of 63.45% exhibited by the sample 7 (5.6% higher than negative control). The seedlings grown in most of the treatments were observed to have either no or positive effect on RWC. Only two samples showed lesser RWC as compared to the control with a minimum of 52.1% exhibited by sample 4 and 17 (5.1% lower than negative control).

3.2. *Phytochemical analysis of* B. napus *plants*

A significant variation in protein content ranging from 0.6% to 60% was observed in the seedlings of all samples treated with river sediment extracts (Table 2). Maximum content was observed against the sample 4 (795.5 μg/10 mg FW) while lowest in the seedling of samples 8 treated plants (499 μg/10 mg FW). Seedlings of all samples also showed higher MDA content comparative to control. Sample 14 was found to have maximum MDA content (107.5 μmol/g of FW) and lowest (43 μmol/g) in the seedlings of the sample 16 (Table 2). Seedlings grown in positive control showed 40.35, 46.41 μM of MDA content per gram FW at the concentration of 500 and 1000 ppm, respectively. A significant variation was observed in phenolics and flavonoid contents of the seedling grown under the treatment of sediments extracts. Among the entire sample, highest total phenolic content was found in the seedlings of the sample 19 (177% increase in comparison with negative control) and lowest content was observed against the sample 3 (45% increase) (Table 2). Positive control at the concentration of 500, 1000 ppm produced enhanced 16% and 40% phenolic content, respectively. Decrease in flavonoids content was observed in the seedlings of the 8 samples and enhanced content was checked against 10 samplesin relation with negative control. Sample 10 was found to have no any effect on total flavonoid content (Table 2).

Seedlings of 17 samples showed increase in chlorophyll a content. Sample 4 was found to have inhibitory effect on chlorophyll a concentration. In case of chlorophyll b, only sample 4 exhibited decrease in concentration while others showed increase in chlorophyll b value. Likewise increase in total chlorophyll content was observed in the seedlings of all samples (significance level $p < 0.05$) except sample 4. Significantly higher content (2.53 mg/g FW) was found against the treatment of sample 10 (Table 3). Eighteen samples were found to have inducing effect toward the production of carotenoids (Table 3). Highest content among all

Table 2
Biochemical evaluation of *Brassica napus* plants treated with River Chenab sediments. The data expressed as mean with standard deviation ($n = 3$). Mean values followed by different small letters are significantly different at the 0.05 probability level using LSD test.

Sites	Total Protein (μg /10 mg FW)	MDA (μM/g FW)	Total Phenolics (μg/g GAE)	Total Flavonoid (μg/g QE)
1	521.5 ± 26.07^{gh}	53.7 ± 1.39^{j}	48.35 ± 1.54^{h}	19.78 ± 0.55^{ef}
2	607.6 ± 30.38^{ef}	61.6 ± 1.6^{i}	85.73 ± 2.74^{b}	25.34 ± 0.7^{bc}
3	639.75 ± 31.98^{de}	67.8 ± 1.76^{h}	51.46 ± 1.64^{g}	19.98 ± 0.55^{ef}
4	795.5 ± 39.77^{a}	99.9 ± 2.59^{c}	70.6 ± 2.25^{e}	21.33 ± 0.59^{de}
5	712.5 ± 35.62^{c}	105.4 ± 2.74^{b}	83.33 ± 2.6^{bc}	27.15 ± 0.76^{b}
6	750.75 ± 37.53^{b}	99.2 ± 2.58^{c}	85.53 ± 2.73^{b}	30.2 ± 0.84^{a}
7	590.75 ± 29.53^{f}	60.2 ± 1.56^{i}	69.5 ± 2.52^{ef}	18.78 ± 0.52^{f}
8	499.5 ± 24.97^{h}	92.8 ± 2.41^{de}	71.54 ± 2.28^{e}	19.89 ± 0.55^{ef}
9	539 ± 26.95^{g}	79.4 ± 2.06^{f}	68.46 ± 2.19^{ef}	17.58 ± 0.49^{f}
10	620.5 ± 31.02^{e}	61.4 ± 1.59^{i}	71.9 ± 2.3^{e}	20.1 ± 0.56^{e}
11	621.5 ± 31.07^{e}	96.4 ± 2.5^{d}	68.69 ± 2.19^{f}	19.65 ± 0.55^{ef}
12	553.75 ± 27.68^{fg}	89.7 ± 2.33^{e}	70.56 ± 2.25^{e}	19.14 ± 0.54^{ef}
13	694.25 ± 34.71^{cd}	104.9 ± 2.72^{ab}	79.9 ± 2.55^{cd}	21.24 ± 0.59^{de}
14	716.25 ± 35.81^{c}	107.5 ± 2.79^{a}	85.04 ± 2.72^{b}	22.5 ± 0.63^{d}
15	542.25 ± 27.11^{g}	49.8 ± 1.29^{k}	81.49 ± 2.6^{c}	15.09 ± 0.42^{g}
16	638.5 ± 31.92^{de}	43 ± 1.11^{l}	83.58 ± 2.67^{bc}	24.9 ± 0.69^{c}
17	672.75 ± 33.63^{d}	70.0 ± 1.82^{g}	78.83 ± 2.52^{cd}	22.84 ± 0.63^{d}
18	711 ± 35.55^{c}	68.1 ± 1.77^{gh}	80.9 ± 2.58^{c}	23.47 ± 0.65^{cd}
19	774.75 ± 38.73^{ab}	100.3 ± 2.6^{c}	98.34 ± 3.14^{a}	25.04 ± 0.70^{bc}
N	496.5 ± 24.82^{h}	38.5 ± 1.2^{m}	35.4 ± 1.13^{j}	20.13 ± 0.56^{e}
p500	518 ± 25.9^{gh}	40.3 ± 1.04^{m}	41.07 ± 1.50^{i}	22.45 ± 0.62^{d}
p1000	591 ± 29.55^{f}	46.4 ± 1.2^{kl}	49.88 ± 1.59^{gh}	26.2 ± 0.733^{b}

Table 3
Chlorophyll and carotenoid contents of *Brassica napus* plants treated with River Chenab sediments. The data expressed as mean with standard deviation ($n = 3$). Mean values followed by different small letters are significantly different at the 0.05 probability level using LSD test.

Sites	Chlorophyll a ($mg\,g^{-1}$ FW)	Chlorophyll b ($mg\,g^{-1}$ FW)	Total Chlorophyll ($mg\,g^{-1}$ FW)	Chl a ratio b	Carotenoid ($mg\,g^{-1}$ FW)
1	1.65 ± 0.03^{b}	0.61 ± 0.006^{a}	2.05 ± 0.05^{b}	2.71	0.13 ± 0.003^{c}
2	0.91 ± 0.02^{g}	0.46 ± 0.01^{cd}	1.11 ± 0.03^{g}	1.96	0.08 ± 0.002^{g}
3	0.65 ± 0.01^{h}	0.31 ± 0.01^{g}	0.83 ± 0.02^{j}	2.09	0.09 ± 0.002^{f}
4	0.23 ± 0.00^{k}	0.09 ± 0^{n}	0.33 ± 0.01^{m}	2.60	0.03 ± 0.0^{k}
5	1.42 ± 0.03^{d}	0.49 ± 0.01^{c}	1.77 ± 0.04^{cd}	3.18	0.13 ± 0.003^{c}
6	1.1 ± 0.02^{f}	0.35 ± 0.01^{f}	1.33 ± 0.03^{e}	3.13	0.16 ± 0.004^{a}
7	0.71 ± 0.01^{h}	0.18 ± 0.0^{l}	0.84 ± 0.02^{h}	3.94	0.12 ± 0.002^{d}
8	0.58 ± 0.01^{i}	0.14 ± 0.01^{m}	0.69 ± 0.02^{k}	4.00	0.11 ± 0.001^{de}
9	0.98 ± 0.02^{fg}	0.27 ± 0.0^{hi}	1.20 ± 0.03^{f}	3.56	0.13 ± 0.003^{c}
10	1.96 ± 0.04^{a}	0.60 ± 0.01^{a}	2.53 ± 0.06^{a}	3.25	0.13 ± 0.003^{c}
11	1.18 ± 0.02^{f}	0.41 ± 0.01^{e}	1.52 ± 0.04^{d}	2.88	0.14 ± 0.004^{b}
12	1.58 ± 0.03^{c}	0.39 ± 0.01^{f}	1.89 ± 0.04^{c}	4.03	0.13 ± 0.003^{c}
13	0.57 ± 0.01^{c}	0.26 ± 0.0^{i}	0.79 ± 0.02^{j}	2.16	0.11 ± 0.002^{de}
14	1.26 ± 0.03^{e}	0.44 ± 0.01^{d}	1.52 ± 0.03^{d}	2.85	0.13 ± 0.003^{c}
15	0.74 ± 0.01^{h}	0.24 ± 0.0^{j}	0.89 ± 0.02^{h}	3.07	0.08 ± 0.002^{g}
16	0.71 ± 0.01^{h}	0.29 ± 0.01^{h}	0.86 ± 0.02^{hi}	2.44	0.12 ± 0.003^{d}
17	0.99 ± 0.02^{fg}	0.58 ± 0.01^{b}	1.23 ± 0.03^{f}	1.70	0.13 ± 0.004^{c}
18	0.73 ± 0.01^{h}	0.21 ± 0.01^{k}	0.89 ± 0.02^{h}	3.54	0.10 ± 0.002^{e}
19	1.01 ± 0.02^{f}	0.39 ± 0.01^{f}	1.28 ± 0.03^{ef}	2.59	0.10 ± 0.003^{e}
N	0.36 ± 0.01^{j}	0.19 ± 0.0^{l}	0.48 ± 0.01^{l}	1.87	0.07 ± 0.001^{h}
p500	0.04 ± 0.003^{l}	0.03 ± 0.0^{o}	0.03 ± 0.0^{n}	1.56	0.05 ± 0.0^{i}
p1000	0.01 ± 0.001^{m}	0.02 ± 0.0^{p}	0.02 ± 0.0^{o}	0.80	0.04 ± 0.001^{j}

samples were found in the seedling of sample 6 (0.6 mg/g FW). While 0.053 and 0.038 mg/g carotenoid contents were observed in the seedling of positive control at 500 and 1000 ppm, respectively.

3.3. *Enzymatic activities of* B. napus *seedlings*

Significant variation in protease, POD and SOD was observed in comparison with control. The significance level was high in case of POD while for protease and SOD significance level and low and moderate, respectively. Seedlings of 16 samples showed protease activity higher than the control with a maximum of 2.94 U/g FW exhibited by sample 4 (Table 4). The remaining seedlings of 3 sediment samples (1, 17 and 18) showed reduction in protease activity. Plantlets of 16 samples showed induction in POD activity and 2 samples i.e. 12, 15 showed decrease in activity. Neither stimulatory nor inhibitory effect on POD activity was observed against the sample 1. In case of SOD, the highest activity in comparison with negative control i.e. 76 nmol/min/g FW was measured in seedlings of sample 4 and lowest was reported against sample 19 as 54.5 nmol/min/g FW activity (Table 4).

4. Discussion

Inadequacy of water resources and the amplified contamination of water and soil adversely affect aquatic ecosystem and agricultural output worldwide. Pakistan is enriched with freshwater resources including streams, canals and rivers which fulfill the needs for irrigation, industrial and domestic purposes (Bhatti and Latif, 2011). Although plants have diverse adaptive mechanisms to cope with different stresses but the understanding of such mechanisms is still incomplete. Ecotoxicity evaluation of sediments of river Chenab was carried out using growth, phytochemical and

Table 4
Enzymatic evaluation of *Brassica napus* plants treated with River Chenab sediments. The data expressed as mean with standard deviation ($n = 3$). Mean values followed by different small letters are significantly different at the 0.05 probability level using LSD test.

Sites	Protease (U/g FW)	POD (nM/min/mg)	SOD (nmol/min/g FW)
1	2.20 ± 0.07^{c}	0.43 ± 0.01^{f}	57.5 ± 1.72^{de}
2	2.66 ± 0.08^{ab}	0.76 ± 0.01^{a}	68.8 ± 2.06^{b}
3	2.67 ± 0.08^{ab}	0.62 ± 0.01^{c}	60 ± 1.8^{d}
4	2.94 ± 0.09^{a}	0.71 ± 0.00^{b}	76 ± 2.28^{a}
5	2.76 ± 0.08^{ab}	0.77 ± 0.00^{a}	69 ± 2.07^{b}
6	2.63 ± 0.08^{ab}	0.69 ± 0.01^{b}	73 ± 2.19^{ab}
7	2.63 ± 0.08^{ab}	0.57 ± 0.00^{de}	60 ± 1.8^{d}
8	2.44 ± 0.06^{b}	0.68 ± 0.00^{b}	68.5 ± 2.05^{b}
9	2.69 ± 0.08^{ab}	0.61 ± 0.01^{d}	61.5 ± 1.84^{d}
10	2.43 ± 0.07^{b}	0.57 ± 0.01^{de}	69 ± 2.07^{b}
11	2.52 ± 0.07^{b}	0.6 ± 0.00^{d}	61.5 ± 1.84^{d}
12	2.41 ± 0.07^{b}	0.42 ± 0.00^{g}	73.5 ± 2.2^{ab}
13	2.82 ± 0.08^{a}	0.63 ± 0.01^{c}	58.5 ± 1.75^{de}
14	2.62 ± 0.08^{ab}	0.61 ± 0.01^{c}	69.5 ± 2.08^{b}
15	2.44 ± 0.07^{b}	0.41 ± 0.00^{g}	61 ± 1.83^{d}
16	2.38 ± 0.07^{b}	0.47 ± 0.00^{f}	72 ± 2.16^{ab}
17	2.20 ± 0.06^{bc}	0.55 ± 0.01^{e}	65 ± 1.95^{c}
18	2.13 ± 0.06^{bc}	0.58 ± 0.01^{d}	56 ± 1.68^{de}
19	2.65 ± 0.08^{ab}	0.56 ± 0.01^{e}	54.5 ± 1.63^{e}
N	2.23 ± 0.07^{bc}	0.43 ± 0.01^{f}	21 ± 2.14^{g}
p500	2.31 ± 0.07^{bc}	0.46 ± 0.00^{f}	34 ± 1.02^{f}
p1000	2.59 ± 0.08^{ab}	0.59 ± 0.01^{d}	56 ± 1.68^{de}

enzymatic bioassays of *B. napus* seedlings germinated in presence of sediments extracts. Ex situ determination of phytotoxicity is an effective tool to determine water quality as it reduces the artifacts related to sample handling and allow a much more realistic exposure.

4.1. Germination, weight and water content of seedlings

Final germination of *B. napus* seeds was recorded on 5th day of seed sowing. All the samples collected from 19 different sites inhibited the seed germination at varying level. Inhibitory effects on plant growth might be due to accumulation of toxic residues in soil sediments of various pesticides like DDTs, heptachlor, endosulfan and others reported from these areas (Eqani et al., 2012a). The organic pollutants induce physical constraints which in turn inhibit germination (Adam and Duncan, 2002). The variation in the rate of germination represents the degree of contamination of that site. The rate of germination was less significant as compared with final germination of *B. napus* seeds. Mean period of final germination (MPFG) was least affected by all the samples. The seeds did not show any variation among the sediments treatments however, in comparison with negative and positive control there was significant response in MPFG. Differences in MPFG among seeds is the crucial factor determining emergence performance both in terms of the rate of emergence, variation, final count and seedling size (Mavi et al., 2010). MPFG is also the mean of the lag period from the start of imbibition to physiological germination (radicle protrusion) which provided an explanation for the link between ageing and MPFG (Khajeh-Hosseini et al., 2009). Pollutants from nearby plants, low dissolved oxygen, chlorinated biphenyls and high OCPs are reported toxic chemicals of this area (Eqani et al., 2011, 2012b, 2013).

The sediments extract not only inhibited seed germination but also put negative on plant length and mass. Sample 4 produced maximum inhibition in RL that was taken from district Khanewal at river Ravi upstream which receives pollutants from municipal, urban, industrial discharges and agricultural runoff from various cities of Punjab i.e. Lahore, Qasoor and Shiekhupura etc. It has been reported that pollutants initially inhibit RL then affect SL of the newly grown seedlings (Araújo and Monteiro, 2005; Chen et al., 2000). The plant growth, in the presence of pollutants, is affected in the following order RL > SL > RG (Song, 2006). The *B. napus* seedlings also showed decrease in RDW when grown in presence of sediments extracts as compared to the control. Results observed in current study show that sediments adversely affected the dry weight rather than fresh weight of the seedlings subscribing that sediments extracts least affected the water holding capacity of plant seedlings. This shows that the mixing of contaminants in water take long way for homogeneity. These findings have reported by others (Kirnak et al., 2001).

Present study shows increase in water content in seedlings of all samples. The significant increase in WC was found in seedlings of 11 samples. Under stress conditions, inhibition of synthesis of various secondary metabolites takes place resulting in decrease in biochemical contents. Consequently the water contents become greater in proportion (Singh et al., 2009). Results show that RWC of *B. napus* leaves was not significantly affected by most of the sediment samples. The samples taken from least contaminated areas (collected from agricultural sites) showed high RWC compared to highly contaminated areas (urban and industrial locations). Among all samples, 16 showed positive effects on RWC of seedlings in comparison with negative control. In case of relative dry weight, sample 4 with a maximum of 11.35% increase as compared to the control was measured that was taken from an industrial drainage area receiving huge amount of pollutants, located in district Jhang.

4.2. Phytochemical analysis of B. napus seedlings

Protein production in plant cell is well documented under metal toxicity, pathogenicity, salinity, drought, heat shock and cold condition (Riccardi et al., 1998; Seki et al., 2001). Enhanced protein content ranged from 0.6% to 60% was observed in the seedlings of all samples. Maximum content was observed against the sample 4 of industrial area district Jhang, while lowest was found in the seedlings of sample 8 of agricultural site. Researchers also reported decrease in total protein content of target species under stress conditions (Costa and Spitz, 1997; Palma et al., 2002) which are contrary to our finding, however it also depends upon type and concentration of stress; duration of toxicity; and even plant/tissue type (Rehman et al., 2014). The variation in protein content may lead to easy evaluation of the impact of stresses on different parameters of biochemistry and physiology of plant.

Malondialdehyde (MDA) is the end product of lipid peroxidation. That is an important marker for assessing cellular damage, injury of cellular membrane and detrimental effects on cells (Cai et al., 2011). *B. napus* plants were found to more sensitive towards sediment samples which could be the base for oxidative damage. Seedlings of all 19 samples exhibited high MDA content. Seedlings grown in most of the samples of agricultural sites with elevated MDA level may be attributed by the sediment contamination with organic and inorganic pollutants. It may contain a huge quantity of pesticides that are being used and the run-off from surrounding fields across the varied stretch of river Chenab.

Phenolics are the group of organic aromatic compounds which are important markers for the evaluation of oxidative stress. Increase in phenolic content was observed in all samples. Among all sample highest content was found in the seedlings of the sample 19 taken from an urban area of district Multan while the lowest content was observed against the sample 3 from an agricultural area. The results of current study are in consistent with findings of (Romero et al., 2004) as the higher polyphenols was found in target plants due to oxidative stress.

Flavonoids are secondary plant metabolites synthesized by plants that are subjected to various stressful conditions like low

nutrient situations, oxidative stress, low temperatures, injury or any infection (Ruiz et al., 2003). They may act as antioxidants through different ways, most likely by free radical scavenging during which the flavonoid substances can break the chain reaction of free radicals. Decrease in flavonoids content was observed in the seedlings of the 8 samples and enhanced content was checked against 10 samples. The highest content was found in the seedlings of sample 6 taken from an urban area of district Jhang that might have produced significant oxidative stress which may indicate the stress generation of sediments for their enhanced production of flavonoids in target plant.

The chlorophyll contents in plants are measured with the aim of evaluating the impact of environmental stress. Being photosynthetic in nature, plants have the greater potential to tolerate environmental and oxidative stresses. However, various characteristics of plants like their physiological and morphological traits may be affected by disturbance in chlorophyll content, growth rate, photosynthetic rate, food storage and respiration rate etc (Valladares and Niinemets, 2008). Increased total chlorophyll content was observed in the seedlings of all samples. Significantly higher content (2.53 mg/g FW) was found against the treatment of sample 10 taken from an agricultural area. Present study correlates with other findings that favorable environmental conditions improve chlorophyll contents in plants (Boonpragob and Nash, 1991). Samples 2 and 4 were found to have inhibitory effects; both belong to the industrial sites of Gujranwala and Jhang, respectively. In case of chlorophyll b only sample 4 of industrial area near Jhang district decreased the quantity while remaining induced increase in chlorophyll content. Mahhou and Dennis (1991) also observed the inhibition of chlorophyll a synthesis against stressed conditions. Presence of higher concentration of toxic substances in the samples of industrial areas may decrease the total chlorophyll content (Jianrong and Qiran, 2009). As the concentration of pollutants in urban and industrial areas are higher than agricultural sites of rural areas where nitrogen and sulphur compounds can serve as nutrients. It is also suggested that chlorophyll synthesis could be stimulated at the agricultural sites due to nitrogen compounds (NO^{3-}, NH^{4+}), which are beneficial to plants (Boonpragob and Nash, 1991). Higher content of chlorophyll a and b in the rural areas could be explained by a fertilizing influence of collected sediment samples from respective sites.

Carotenoids are important component of plants required for photo protection, photosynthesis, and phyto-hormones. These are capable to absorb adequate light for photosynthesis, protect the membrane and proteins from photo oxidative damage. Carotenoids quench triplet chlorophyll; scavenge ROS like singlet oxygen which damage membranes and proteins, thereby acting as antioxidants (Niyogi 1999; Howitt and Pogson, 2006; Bailey and Grossman, 2008; Alboresi et al., 2011). In the present study, eighteen samples were found to have inducing effect towards the production of carotenoids. Highest content among all samples were found in the seedling of sample 6 that was taken from an industrial site of district Jhang. Enhanced carotenoids content may be caused by oxidative stress of contaminated area. Chaves et al. (2002) and Wang et al. (2004) also reported the elevated carotenoids under stressed conditions. These samples may contain various organic and inorganic pollutants like hazardous chemicals of waste effluents and heavy metals released from industries which can damage the entire synthetic machinery of plant. The enhancement of carotenoids content may also be correlated with the improved tolerance of target plant against various abiotic environmental stresses (Zaefyzadeh et al., 2009).

4.3. *Enzymatic activities in* B. napus *seedlings*

Proteases are vital enzymes in regulating various biological processes like detecting stress conditions, pests, pathogens and numerous biochemical mechanisms to combat with oxidative and environmental stresses. They are involved in various aspects of the life cycle of plant ranging from the stored protein mobilization during germination of seed to the disruption of metabolism and initiation of cell death (Milošević et al., 2010) and protein mobilization during chemical or environmental stresses (Domash et al., 2008). Most of the samples from agricultural area with reported agricultural pollution and presence of obsolete pesticides (Eqani et al., 2012c) showed maximum protease activity in the grown seedlings. The highest protease activity (2.94 U/g FW) was exhibited by sample 4 taken from an industrial area of densely populated District Jhang. The seedlings of only 3 sediment samples showed reduction in protease activity with minimum of 2.135 U/g FW measured in the seedling of sample 18 from an agricultural area of district Multan. Possibly, these pollutants may have inhibitory and damaging effect on the metabolic processes or they could interfere with the process of enzymatic activity in target plant (Jajoo et al., 2014).

Peroxidases (POD) are important enzymes of animals, yeast, microorganisms and plants performing various functions in cell, such as association in cell wall synthesis, lignification and defense mechanism during infection (Pourcel et al., 2007; Scandalios, 2005). Plantlets of 16 samples showed induction in POD activity and 2 samples i.e. 12, 15 showed decreased activity. One sample was found to have no any inducing or inhibitory effect for POD when compared with negative control. Diverse nature of pollutants was observed in these areas reflecting the wide scale contamination in the neighboring fields. Mazorra et al. (2002) also reported the enhanced antioxidant activities and tolerance in stressed condition.

Superoxide dismutases (SOD) play crucial role in antioxidative defense mechanism. An increase in SOD activity of seedlings in response to all sediment samples was observed. A significant difference in relative induction of SOD was also observed. Doganlar (2012) observed increased level of SOD against environmental stress. In present study, the highest percent relative induction i.e. 76% was measured in seedlings of sample 4 from an industrial area of District Jhang and lowest was reported against sample 19 as 54.5% activity taken from an agricultural site of Multan. It can be proposed that SOD can be utilized for screening oxidative resistant materials of plant as an indirect selection criterion (Zaefyzadeh et al., 2009). An increase in SOD activity also attributes to the increased production of active oxygen species (Rehman et al., 2014). Our results correlates with other studies reporting increase in SOD activity in various species in response to drought, salt and oxidative stresses (Gunes et al., 2008; Rajabi et al., 2012). The current study suggest that superoxide dismutase and peroxidase isozymes can act as useful markers in the analysis of metabolic regulations and gene functions, including stress-tolerance characteristics. However, the level of oxidative scavengers in response of stressful conditions depends on the type of target tissue, species, concentration and type of contamination (San Miguel et al., 2012; Mittler 2002).

Current study comprised of the exploration of phytochemical, enzymatic variation and growth modulation effects of sediments of river Chenab on *B. napus*. The sediments of Chenab River produced significant effect on morphology, physiology and biochemistry of *B. napus* plants. Sediment samples either collected from agriculture,

rural or industrial areas may contain organic and metallic pollutants or accumulation of fertilizers that induced stress on the plants. Moreover the sediments samples used are a complex chemical mixture which increases the difficulty of interpretation of the results. Future studies should determine the physiological state of the seeds that germinated in the presence of the pollutants contained in these sediments samples. Therefore, more attention should be paid to control these pollutants in the Chenab River.

Conflict of interest

The authors declare no competing financial interest.

References

Adam, G., Duncan, H., 2002. Influence of diesel fuel on seed germination. Environ. Pollut. 120, 363–370.

Alboresi, A., Dall'Osto, L., Aprile, A., Carillo, P., Roncaglia, E., Cattivelli, L., Bassi, R., 2011. Reactive oxygen species and transcript analysis upon excess light treatment in wild-type *Arabidopsis thaliana* vs a photosensitive mutant lacking zeaxanthin and lutein. BMC Plant Biol. 11, 62.

Apel, K., Hirt, H., 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55, 373–399.

Araújo, A.S.F., Monteiro, R.T.R., 2005. Plant bioassays to assess toxicity of textile sludge compost. Sci. Agricola. 62, 286–290.

Bailey, S., Grossman, A., 2008. Photoprotection in Cyanobacteria: regulation of light harvesting. Photochem. Photobiol. 84, 1410–1420.

Bailly, C., Benamar, A., Corbineau, F., Côme, D., 1996. Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. Physiol. Plant. 97, 104–110.

Bhatti, M.T., Latif, M., 2011. Assessment of water quality of a river using an indexing approach during the low-flow season. Irrig. Drain. 60, 103–114.

Boonpragob, K., Nash, T.H., 1991. Physiological responses of the lichen *Ramalina menziesii* Tayl. to the Los Angeles urban environment. Environ. Exp. Bot. 31, 229–238.

Bor, M., Özdemir, F., Türkan, I., 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritima* L. Plant Sci. 164, 77–84.

Bornette, G., Puijalon, S., 2011. Response of aquatic plants to abiotic factors: a review. Aquat. Sci. 73, 1–14.

Cai, F., Mei, L.J., An, X.L., Gao, S., Tang, L., Chen, F., 2011. Lipid peroxidation and antioxidant responses during seed germination of *Jatropha curcas*. Int. J. Agric. Biol. 13, 25–30.

Chang, C., Yang, M., Wen, H., Chern, J., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 10, 178–182.

Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osorio, M.L., Carvalho, I., Faria, T., Pinheiro, C., 2002. How plants cope with water stress in the field: photosynthesis and growth. Ann. Bot. 89, 907–916.

Chen, L., Lin, C.L., Kao, C., Lin, C.C., Kao, C.H., 2000. Copper toxicity in rice seedlings: changes in antioxidative enzyme activities H_2O_2 level and cell wall peroxydase activity in roots. Bot. Bull. Acta Sin. 41, 99–103.

Costa, G., Spitz, E., 1997. Influence of cadmium on soluble carbohydrates, free amino acids, protein content of in vitro cultured *Lupinus albus*. Plant Sci. 128, 131–140.

Doganlar, Z.B., 2012. Physiological and genetic responses to pesticide mixture treatment of *Veronica beccabunga*. Water Air Soil Pollut. 223 (9), 6201–6212.

Domash, V.I., Sharpio, T.P., Zabreiko, S.A., Sosnovskaya, T.F., 2008. Proteolytic enzymes and trypsin inhibitors of higher plants under stress conditions. Rus. J. Bio. Chem. 34, 318–322.

Dupuy, J., Ouvrard, S., Leglize, P., Sterckeman, T., 2015. Morphological and physiological responses of maize (*Zea mays*) exposed to sand contaminated by phenanthrene. Chemosphere 124, 110–115.

Eqani, S.A.M.A.S., Malik, R.N., Mohammad, A., 2011. The level and distribution of selected organochlorine pesticides in sediments from River Chenab, Pakistan. Environ. Geochem. Health 33, 33–47.

Eqani, S., Malik, R.N., Katsoyiannis, A., Zhang, G., Chakraborty, P., Mohammad, A., Jones, K.C., 2012a. Distribution and risk assessment of organochlorine contaminants in surface water from River Chenab, Pakistan. J. Environ. Monit. 14, 1645–1654.

Eqani, S.A.M.A.S., Malik, R.N., Zhang, G., Mohammad, A., Chakraborty, P., 2012b. Polychlorinated biphenyls (PCBs) in the sediments of the River Chenab, Pakistan. Chem. Ecol. 128, 327–339.

Eqani, S.A.M.A.S., Malik, R.N., Alamdar, A., Faheem, H., 2012c. Status of organochlorine contaminants in the different environmental compartments of Pakistan: a review on occurrence and levels. Bull. Environ. Contam. Toxicol. 88, 303–310.

Eqani, S.A.M.A.S., Malik, R.N., Cincinelli, A., Zhang, G., Mohammad, A., Qadir, A., Rashid, A., Bokhari, H., Jones, K.C., Katsoyiannis, A.I., 2013. Uptake of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) by river water fish: the case of River Chenab. Sci. Total Environ. 450, 83–91.

Foyer, C.H., Noctor, G., 2005. Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ. 28, 1056–1071.

Giavalisco, P., Kapitza, K., Kolasa, A., Buhtz, A., Kehr, J., 2006. Towards the proteome of *Brassica napus* phloem sap. Proteomics 6, 896–909.

Gunes, A., Pilbeam, D.J., Inala, A., Coban, S., 2008. Influence of silicon on sunflower cultivars under drought stress, I: growth, antioxidant mechanisms, and lipid peroxidation. Commun. Soil Sci. Plant Anal. 39, 1885–1903.

Haq, I.U., Ullah, N., Bibi, G., Kanwal, S., Ahmad, M.S., Mirza, B., 2012. Antioxidant and cytotoxic activities and phytochemical analysis of euphorbia wallichii root extract and its fractions. Iran. J. Pharm. Res. IJPR. 11, 241.

Howitt, C.A., Pogson, B.J., 2006. Carotenoid accumulation and function in seeds and non-green tissues. Plant Cell Environ. 29, 435–445.

Hudson-Edwards, K.A., Macklin, M.G., Jamieson, H.E., Brewer, P.A., Coulthard, T.J., Howardd, A.J., Turner, J.N., 2003. The impact of tailings dam spills and clean-up operations on sediment and water quality in river systems: the Ríos Agrio–Guadiamar Aznalcóllar, Spain. App. Geochem. 18, 221–239.

Ingersoll, C.G., MacDonald, D.D., Brumbaugh, W.G., Johnson, B.T., Kemble, N.E., Kunz, J.L., May, T.W., Wang, N., Smith, J.R., Sparks, D.W., Ireland, D.S., 2002. Toxicity assessment of sediments from the Grand Calumet River and Indiana Harbor Canal in northwestern Indiana, USA. Arch. Environ Cont. Toxicol. 43, 156–167.

Jajoo, A., Mekala, N.R., Tomar, R.S., Grieco, M., Tikkanen, M., Aro, E.M., 2014. Inhibitory effects of polycyclic aromatic hydrocarbons (PAHs) on photosynthetic performance are not related to their aromaticity. J. Photochem. Photobiol. 137, 151–155.

Jianrong, X., Qiran, T., 2009. Early stage toxicity of excess copper to photosystem II of *Chlorella pyrenoidosa*-OJIP chlorophyll a fluorescence analysis. J. Environ. Sci. 21, 1569–1574.

Kandil, A.A., Sharief, Ali E., Nassar, E.S.E., 2012. Response of some rice (*Oryza sativa* L.) cultivars to germination under salinity stress. Int. J. Agric. Sci. 4, 272–277.

Khajeh-Hosseini, M., Lomholt, A., Matthews, S., 2009. Mean germination time in the laboratory estimates the relative vigour and field performance of commercial seed lots of maize (*Zea mays* L.). Seed Sci. Technol. 37, 446–456.

Kirnak, H., Kaya, C., Ismail, T.A.S., Higgs, D., 2001. The influence of water deficit on vegetative growth, physiology, fruit yield and quality in eggplants. Bulg. J. Plant Physiol. 27, 34–46.

Lagrimini, L.M., 1991. Wound-induced deposition of polyphenols in transgenic plants overexpressing peroxidase. Plant Physiol. 96, 577–583.

Liu, H., Weisman, D., Ye, Y.B., Cui, B., Huang, Y.H., Colon-Carmona, A., Wang, Z.H., 2009. An oxidative stress response to polycyclic aromatic hydrocarbon exposure is rapid and complex in *Arabidopsis thaliana*. Plant Sci. 176, 375–382.

Long, E.R., MacDonald, D.D., Smith, S.L., Calder, F.D., 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ. Manage. 19, 81–97.

Macías, F.A., Castellano, D., Molinillo, J.M.G., 2000. Search for a standard phytotoxic bioassay for allelochemicals. Selection of standard target species. J. Agric. Food Chem. 48, 2512–2521.

Mahhou, A., Dennis, F., 1991. Changes in protein profiles in peach embryos during the breaking of dormancy by chilling. Hort. Sci. 26, 733.

Malik, R.N., Nadeem, M., 2011. Spatial and temporal characterization of trace elements and nutrients in the Rawal Lake Reservoir, Pakistan using multivariate analysis techniques. Environ. Geochem. Health 33, 525–541.

Mavi, K., Demir, I., Matthews, S., 2010. Mean germination time estimates the relative emergence of seed lots of three cucurbit crops under stress conditions. Seed Sci. Technol. 38, 14–25.

Mazorra, L.M., Nunez, M., Hechavarria, M., Coll, F., Sánchez-Blanco, M.J., 2002. Influence of brassinosteroids on antioxidant enzymes activity in tomato under different temperatures. Biol. Plant. 45, 593–596.

Milošević, M., Vujaković, M., Karagic, D., 2010. Vigour tests as indicators of seed viability. Genetika 42, 103–118.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7, 405–410.

Nayyar, H., Gupta, D., 2006. Differential sensitivity of C 3 and C 4 plants to water deficit stress: association with oxidative stress and antioxidants. Environ. Exp. Bot. 58, 106–113.

Niyogi, K.K., 1999. Photoprotection revisited: genetic and molecular approaches. Ann. Rev. Plant Biol. 50, 333–359.

Palma, J.M., Sandalio, L.M., Corpas, F.J., Romero-Puertas, M.C., McCarthy, I., delRío, L.A., 2002. Plant proteases, protein degradation, and oxidative stress: role of peroxisomes. Plant Physiol. Biochem. 40, 521–530.

Pereda-Miranda, R., Mata, R., Anaya, A.L., Wickramaratne, D.B.M., Pezzuto, J.M., Kinghorn, A.D., Tricolorin, A., 1993. Major phytogrowth inhibitor from *Ipomoea tricolor*. J. Nat. Prod. 56, 571–582.

Pourcel, L., Routaboul, J.M., Cheynier, V., Lepiniec, L., Debeaujon, I., 2007. Flavonoid oxidation in plants: from biochemical properties to physiological functions. Trends Plant Sci. 12, 29–36.

Rajabi, S., Karimzadeh, G., Ghanati, F., Asilan, K.S., 2012. Salt-induced changes of antioxidant enzymes activity in winter canola (*Brassica napus*) cultivars in growth chamber. J. Plant Physiol. Breed. 2, 11–21.

Rehman, R.U., Zia, M., Abbasi, B.H., Lu, G., Chaudhary, M.F., 2014. Ascorbic acid and salicylic acid mitigate NaCl stress in Caralluma tuberculata Calli. App. Biochem. Biotechnol. 73, 968–979.

Riccardi, F., Gazeau, P., de Vienne, D., Zivy, M., 1998. Protein changes in response to progressive water deficit in maize quantitative variation and polypeptide identification. Plant Physiol. 117, 1253–1263.
Romero, C., Brenes, M., Yousfi, K., García, P., García, A., Garrido, A., 2004. Effect of cultivar and processing method on the contents of polyphenols in table olives. J. Agric. Food Chem. 52, 479–484.
Rouhier, N., Koh, C.S., Gelhaye, E., Corbier, C., Favier, F., Didierjean, C., Jacquot, J.P., 2008. Redox based anti-oxidant systems in plants: biochemical and structural analyses. Biochim. Biophys. Acta 1780, 1249–1260.
Ruiz, J.M., Rivero, R.M., López-Cantarero, I., Romero, L., 2003. Role of Ca^{2+} in the metabolism of phenolic compounds in tobacco leaves (*Nicotiana tabacum* L.). Plant Growth Reg. 41, 173–177.
San Miguel, A., Faure, M., Ravanel, P., Raveton, M., 2012. Biological responses of maize (*Zea mays*) plants exposed to chlorobenzenes Case study of monochloro-1, 4-dichloro-and 1, 2, 4-trichloro-benzenes. Ecotoxicology 21 (2), 315–324.
Scandalios, J., 2005. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. Braz. J. Med. Biol. Res. 38, 995–1014.
Seki, M., Narusaka, M., Abec, H., Kasugac, M., Yamaguchi-Shinozakic, K., Carnincid, P., Hayashizakid, Y., Shinozakia, K., 2001. Monitoring the expression pattern of 1300 Arabidopsis genes under drought and cold stresses by using a full-length cDNA microarray. Plant Cell. 13, 61–72.
Singh, N., Singh, D., Singh, A., 2009. Modification of physiological responses of water stressed *Zea mays* seedlings by leachate of *Nicotiana plumbaginifolia*. Gen. App. Plant Physiol. 35, 51–63.
Song, X., 2006. Soil-based eco-toxicity of diesel oil contamination to seed germination and seedling growth of wheat. J. Agro.-Environ. Sci. 25, 554–559.
Stoeva, N., Bineva, T., 2003. Oxidative changes and photosynthesis in oat plants grown in As-contaminated soil. Bulg. J. Plant Physiol. 29, 87–95.
Turker, A.U., Camper, N., 2002. Biological activity of common mullein, a medicinal plant. J. Ethnopharmacol. 82, 117–125.
Turner, N.C., 1986. Crop water deficits: a decade of progress. Adv. Agron. 39, 1–51.
Ullah, N., Haq, I.U., Mirza, B., 2013. Phytotoxicity evaluation and phytochemical analysis of three medicinally important plants from Pakistan. Toxicol. Indus. Health, 748233712472527.
Valladares, F., Niinemets, U., 2008. Shade tolerance, a key plant feature of complex nature and consequences. Ann. Rev. Ecol. Evol. Syst., 237–257.
Wang, W., Vinocur, B., Shoseyov, O., Altman, A., 2004. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci. 9, 244–252.
Zaefyzadeh, M., Quliyev, R.A., Babayeva, S.M., Abbasov, M.A., 2009. The effect of the interaction between genotypes and drought stress on the superoxide dismutase and chlorophyll content in durum wheat landraces. Turk. J. Biol. 33, 1–7.