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RESEARCH ARTICLE

Role of transitory starch on growth, development and metal accumulation of *Triticum aestivum* cultivars grown under textile effluent fertilization

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Abstract
Accumulation of transitory starch in leaves is an environment-dependent multifaceted process affected through stress caused by nutrient deficiency or excess of heavy metals in growing medium. On the other hand, textile effluent is one of the major pollution causing industrial waste due to the presence of heavy metal and organic contaminants. Besides the presence of higher pollution load, this effluent also contains some minerals essential for plant growth and metabolism and can serve as source of nutrients to plants. In presented experiment, a mesocosm study was conducted to evaluate the phenotypic, biochemical performance and trace element status of *Triticum aestivum* (cv. LOK-101 and GW-496) cultivars in response to transitory starch activity grown under textile effluent fertilization. Improved activity of transitory starch under textile effluent fertilization deals with plant growth by providing carbon in the form of soluble sugar. Study also finds a strong correlation of photosynthetic pigments, carbohydrates and plant biomass to transitory starch. As expected, the elemental concentration (Zn, Cu, Mn, Fe, Co, Pb, Cd, and As) in plants increased with increasing dose of textile effluent. The study concluded that the transitory starch is one of the key components in plant leaves that regulate plant growth under stress condition. Furthermore, the study also concluded that the lower dose of textile effluent significantly favours growth and nutrient status of plants without any negative impact. Therefore, the application of lower concentration of textile effluent as basal dose in agriculture may serve as source of nutrient/micronutrient to plants and also can be a sustainable way for effluent management.

Keywords Transitory starch · Textile effluent · *Triticum aestivum* · Principle component analysis · Transition factor · Enrichment factor

Introduction

Physiology of transitory starch has become a major topic of research these days with increasing evidence of its involvement in response to stress. Growth and development of plant required high energy during stress conditions and degraded starch is the available carbon source under such conditions. Among the two types of starch, i.e. storage starch and transitory starch (Pfister and Zeeman 2016), transitory starch is primary product of photosynthesis, composed of glucose polymer such as amylose and amylopectin. This particular starch was synthesized using a fraction of the fixed CO_2 and temporally stored at foliar chloroplast in the form of insoluble granules (Orzechowski 2008). This synthesis is seen as an intermediate component of cyclic gluconeogenic pathway and depends on the rate of photosynthesis (Baroja-Fernández et al. 2001; Santelia and Lunn 2017). Biosynthesis and degradation of transitory starch supports the plant health, growth and height, and development by providing the sugar to sustain metabolisms in leaf organs during dark when the process of photosynthesis does not occur (Geigenberger 2011). Transitory starch also plays an important role in opening and closing of stomata (Santelia and Lunn 2017). Changes in starch content during stress condition are clear indicators of a variety of plant developmental processes

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which protects the plant growth by synthesizing the sugar and proline (Wei et al. 2015).

Minerals such as Zn, Mg and Fe required for plant health and the deficiency of any of these minerals strongly influence plant developmental processes. Deficiency of essential minerals in soil also affects photosynthetic apparatus structure and functions, including PSII photochemistry (Kalaji et al. 2018). Photosynthesis provides material and energy for the growth and development of plants in the form of transitory starch, in which chlorophyll plays a significant role (Kalaji et al. 2018). Excessive concentration of salt or metals within plant body allows distribution through phloem along with nutrients to the plant tissues, interrupting the proper light penetration which further ceases the photosynthetic activity through chlorophyll content (Manios et al. 2003; Kalaji et al. 2018) and decrease in photosynthetic activity indirectly affects the synthesis and accumulation strategy of transitory starch. Age of plant also affects the plant health. Metabolic activity of plant and degradation of starch partitioning to sucrose and reducing sugar started to slow down as plant matures; thus, the plant responses to the stress caused by pollution or plant age are correspondingly different (Wolf 1993; Page and Feller 2015).

India has a large network of industries of varying capacity that are distributed throughout the country. Textile industry is considered to be one of the world's most pollution-generating industries and the estimated discharge of wastewater from some of the textile industries in India is 25 KLD (kilo litres per day) (Gronwall and Jonsson 2017). Textile effluents contain organic dyes characterized by strong colour, highly fluctuating pH, high COD, BOD, sulphide, TS, TDS and ammonical nitrogen (Kaushik et al. 2005; Gronwall and Jonsson 2017), although the effluent characteristics vary based on many factors like the raw materials used, process involved, products manufactured, geographical conditions, and so forth (Gronwall and Jonsson 2017). Despite consisting high concentration of heavy metals and organic compounds, some essential metals, e.g. Fe, Cu, Mn, Zn, Ni, are necessary for plant metabolism as enzyme activators or regulators are also present in textile effluent (Kaushik et al. 2005; Hassan et al. 2013). As it contains various trace elements and nutrients, the application of limited concentration of effluents could support plant growth and crop production (Singh and Rathore 2018). Thus, application of textile effluent with dilution as a basal dose can offer a multidimensional advantage that satisfies the need of micronutrients to the plants at the same time reduces the direct inputs of chemical fertilizers and minimizing its pollution loads in the soil.

Many reports are available for application of different industrial wastewater and effluent in agriculture as a nutrient source under water scarcity condition. Their pros and cons are also reported due to long-term utilization. Very few reports are available for controlled use of textile effluents as fertilizers. Furthermore, the accumulation of transitory starch in leaves is an environment-dependent multifaceted process and is not known about the role of transitory starch for growth, development and nutrient uptake by plants under textile effluent fertilization. Therefore, the study present here is aimed to evaluate the role of transitory starch in plant growth, development and nutrient status of two cultivars of *Triticum aestivum* (cv. LOK-101 and GW-496) grown under textile effluent fertilization. We hypothesized that the (1) plant growth and development directly correlate with transitory starch content, (2) transitory starch content depends on the nutrient status of the growing medium, and the textile effluent concentration control the transitory starch accumulation and heavy metal uptake by plants.

Materials and methods

Plant material and experimental design

Genetically uniformed seeds of wheat (*Triticum aestivum*) cultivars LOK-101 and GW-496 are obtained from Beej Bhavan, Govt. of Gujarat, Gandhinagar. Healthy seeds were surface sterilized as described in Singh and Rathore (2018) and sown in total 30 pots (15 for each cultivar) of 19 cm (top diameter) × 18 cm (height). The pots were filled with equal amounts (5 kg pot^{-1}) of slight alkaline (pH 7.4) sandy loam soil of medium fertility (NPK value of used soil was 236.31, 78.63, 118.54 kg ha^{-1}, respectively). Trace element value of the experimental soil before addition of textile effluent was Fe 60.6 ± 0.11, Zn 0.21 ± 0.01, Mn 1.28 ± 0.04, As 0.0028 ± 0.21, Cd 0.0 ± 0.0, Co ND, Al 92.8 ± 1.03, Ni 0.30 ± 0.01, Pb 0.2 ± 0.003 (all values are mg kg^{-1} of soil). Ten seeds/pot of each cultivar were sown in each pot. Four dilutions of textile effluent (10%, 20%, 40% and 60% v/v, i.e. TEF1, TEF2, TEF3 and TEF4, respectively) were applied to soil as the basal fertilizer dose for micronutrient supplementation. No other fertilizer was added in the soil. A control set (without textile effluent fertilization, TEF0) was also maintained for comparison. Ground water was applied for irrigation purpose in all treatments as required. After germination, pots were thinned to six seedlings per pot in all the pots except for those where number of germinated seeds was six or less than six. Further thinning was conducted at each sampling period to provide suitable growing space. The experiment was conducted from November to March. Pots were kept in field condition after sowing the seeds. The climatic condition during the study was found tropical according to the weather report (https://www.weatheronline.in). Minimum temperature during the experimental period was 11 °C, while the maximum temperature was ranging between 31 and 44 °C at the time of harvesting. The average humidity during the study was 45.6% and the day length was 10.42 (during first sampling) to 12.03 (at the time of harvesting).

Sampling of plants for growth and biochemical analysis was carried out at 4 different growth stages, i.e. germination (GrS), vegetative (VS), reproductive (RS) and maturity (MS) of plant. Leaves were sampled at 10:00 h for transitory starch and biochemical analysis at every growth stage. The pots were arranged in completely randomized design, in a 5 × 2 factorial scheme (5 different treatment including control and 2 wheat cultivars), with three replication of each treatment, in total 30 experimental units. Growth stage of plants and treatments were considered as factors.

Effluent collection and characterization

Textile effluent originating from the Mangalam textile industry was collected before treatment from a Green Environment CETP located in Vatwa, Ahmedabad (India). The effluents were stored at 4 °C to avoid changes in its characteristics during storage. Physiochemical and elemental characteristics of collected textile effluent (used in the present study) was pH 6.86 ± 0.1; EC (μS cm^{-1}) 438 ± 0.43; BOD$_5$ (at 20 °C) (mg l^{-1}) 688 ± 1.08; Fe (mg l^{-1}) 1.2 ± 0.063; Zn (mg l^{-1}) 2.16 ± 2.26; Mn (mg l^{-1}) 1.1 ± 0.05; Cr (mg l^{-1}) 4.22 ± 2.34; Pb (mg l^{-1}) ND; Cd (mg l^{-1}) 1.3 ± 0.004; Cu (mg l^{-1}) 1.3 ± 0.002) as described in our previous report (Singh and Rathore 2018).

Plant growth analysis

The simple index of plant growth is the rate of changes which is measured as specific leaf weight (SLW), absolute growth rate (AGR) and total plant biomass (g). Plants were randomly harvested from each treatment including control of both cultivars. The plants were thoroughly washed by distilled water and separated into leaf, stem and root for measurement of length, biomass and leaf area. For biomass, plant parts were oven dried at 80 °C until a constant weight was achieved. SLW of the wheat cultivars were calculated by a formula described by Syvertsen et al. (1980).

$$\mathrm{SLW}\left(\mathrm{g\ cm}^{-2}\right) = [\mathrm{LDW/LA}]$$

where LDW is the leaf dry weight (g) and LA is leaf area (cm^{-1}) respectively.

The absolute growth rate (AGR) was calculated according to Radford (1967) with little modification as follows:

$$\mathrm{AGR}\left(\mathrm{cm\ day}^{-1}\right) = (\mathrm{H}_2\text{–}\mathrm{H}_1)/(\mathrm{T}_2\text{–}\mathrm{T}_1)$$

where H1 and H2 are the total plant lengths (cm) at times (days) T1 and T2, respectively.

Determination of photosynthetic activity

Photosynthetic activity was determined from 0.5 g of leaf from each treatment including control. Leaves were homogenized with 10 mL of chilled 80% acetone and the extract was centrifuged at 5000× for 10 min. Obtained supernatant was collected and make up to 10 mL with 80% acetone. The absorbance of supernatant was read at 645 and 663 nm considering 80% acetone as blank. Estimation of chlorophyll ‘*a*’ and ‘*b*’, Chl *a*/*b* ratio and total chlorophyll was calculated by the formula described by Goodwin (1976).

$$\text{Chlorophyll } a\left(\mathrm{mg\ g}^{-1}\mathrm{fw}\right) = 12.7(\mathrm{OD\ 663})\text{−}2.69(\mathrm{OD\ 645})$$

$$\text{Chlorophyll } b\left(\mathrm{mg\ g}^{-1}\mathrm{fw}\right) = 22.9(\mathrm{OD\ 645})\text{−}4.68(\mathrm{OD\ 663})$$

$$\text{Total Chlorophyll}(a+b)\left(\mathrm{mg\ g}^{-1}\mathrm{fw}\right) = 20.2(\mathrm{OD\ 645}) + 8.02(\mathrm{OD\ 663})$$

$$\text{Chlorophyll a/b ratio} = \frac{\text{Chlorophyll}'a'}{\text{Chlorophyll}'b'}$$

Determination of protein and proline

Protein was estimated by leaf extraction in 0.2 M phosphate buffer of pH 7 following the method of Lowry et al. (1951). Amount of protein present in the samples was expressed with the bovine serum albumin (BSA) as standard in microgram per millilitre.

Proline content was estimated by extracting the leaf sample in 10 mL sulfosalicylic acids and supernatant was used for acid-ninhydrin test (Bates et al. 1973) and expressed as milligram proline per gram FW of leaf.

Determination of soluble sugar, reducing sugar and transitory starch

For determination of carbohydrate, 100 mg of leaf sample was crushed with 80% chilled ethanol followed by centrifugation at 6000× for 5 min. The supernatant was used for soluble and reducing sugars. Soluble sugar was estimated following the anthrone reagent method described by Dubois et al. (1951). Reducing sugar was analysed using DNS reagent (dinitrosalicylic acid) as explained by Breuil and Saddler (1985). Sediment of extract that filtered in sugar content was used for starch determination. The remaining insoluble material was washed twice with ethanol 80% and the residual pellet was treated with 1 M HCL for 2 h at 95 °C for starch hydrolysis. Starch was determined by spectrophotometer according to Jarvis and Walker (1993).

Trace metal analysis

Oven-dried plant parts were used for the wet acid digestion (HNO_3:$HClO_4$:H_2SO_4 10:4:1, v/v, mixture) to determine the trace metal concentration in plant tissue. The Zn, Fe, Mn, Cu, Co, As, Cd and Pb contents in the solution were estimated employing a Perkin-Elmer (Analyst Model Optima 5300 V) plasma atomic emission spectroscopy (ICP-OES). The heavy metal content was expressed as milligram per kilogram dw of the plant tissues.

Data processing and statistical analysis

All the experimental data were analysed in three replication using SPSS program (IBM version 17.0). Two-way analysis of variance (ANOVA) was performed on biochemical activity. Growth stage and textile effluent fertilization (TEF) were considered as independent variables, whereas one-way analysis of variance has been implemented on the trace/heavy metal accumulation by plant tissues. Significant differences in means were separated by the Tukey's multiple range test.

At the vegetative growth stage of plant, bivariate analysis was applied to check the correlation (Pearson correlation coefficient) between transitory starch and biochemical parameters (chlorophyll, proline, protein, reducing sugar and soluble sugar contents) using Excel 2010 to evaluate the role of transitory starch in plant growth and development. Relationship between trace elements in leaves and transitory starch at maturity stage of the plant was also evaluated to estimate the possible effects of trace elements on transitory starch. The principle component analysis (PCA) was performed in order to determine the relationship among characteristics as well as similarity and differences among both the cultivars (LOK-1 and GW-496) under textile effluent fertilization (0% TEF, 10% TEF, 20% TEF, 40% TEF and 60% TEF) at different growth stage (GrS, VS, RS and MS). Standardized PC1 and PC2 scores were plotted using Past3 software (2015).

The metal enrichment factor (EF) was obtained following the formula: EF = concentration of metals in soil or plant tissues from textile effluent/concentration of metals in soil or plant tissues. Accordingly, EF value in plant tissues was defined as $EF < 2$, deficient; $2 < EF < 5$, moderate; $5 < EF < 20$, significant; $20 < EF$ very high. Translocation factor (TF) is obtained through the formula: TF = concentration of metal in aerial part/concentration of metal in root. The coefficient is used to evaluate the ability of plant and their condition to transfer heavy metals to their aerial parts from root in response to their plant growth.

Results and discussion

Textile effluent used in the present study contains non-essential metals (Cd, As, Pb) which might affect the cellular activity of plants to interrupt their growth at higher concentration. However, textile effluent was found to be rich in various mineral and trace metals required for plant physiological activity at lower concentration (Singh and Rathore 2018).

Effects of textile effluent fertilization on morphological traits

Morphological characteristics of wheat cultivars responded significantly to textile effluent fertilization and age of plants (Figs. 1 and S1 and Table 1). Textile effluent application exhibited highest AGR value in TEF2 followed by TEF1 at all growth stages of plants (Fig. 1(a)). Increasing the age of plant decreased AGR value significantly, and this trend followed in all the treatments. SLW was highest in TEF2 while plant age showed slight negatively affecting all the fertilization treatments and control (Fig. 1(b)). Textile effluent helps to increase the plant growth and development to maintain the soil properties through the pH, EC and by providing the organic matter and micronutrients in the limited range at lower concentration (Yaseen et al. 2017; Singh and Rathore 2018). While at higher concentration it may affect the plant growth and lower the SLW and AGR by cellular malfunctioning through imbalance of ROS level and antioxidative response, and deficiency or improper supply of nutrients required for plant growth as in the case of control treatment of the present experiment which lowers the AGR and SLW (Walker et al. 2001). Smaller initial size has a larger relative growth rate as in our findings of decreasing the AGR with increasing the plant age (Rees et al. 2010). Typical outcome of high AGR value could be the reason of high distribution to photosynthesis tissue, while lower AGR described the high allocation to root (Poorter et al. 2012).

Plants grown under textile effluent had positive effects on plant biomass. TEF1 and TEF2 showed a significant higher plant biomass compared with other treatments of both cultivars (Fig. 1(c)). The result attributed due to the readily available plant nutrients supplied from the application of textile effluents (Kaushik et al. 2005) and also the uptake of nutrients by the plant cultivars (Khan et al. 2011; Hassan et al. 2013).

Effects of textile effluents on photosynthetic pigments, protein and proline

Compared with the TEF0, fertilization with lower dose of textile effluents had promoting effects in total chlorophyll and chlorophyll *a*/*b* ratio (Fig. 2(a) and (b)), although higher concentration of textile effluent significantly decreases the *a*/*b* ratio. TEF0 showed the lowest value for

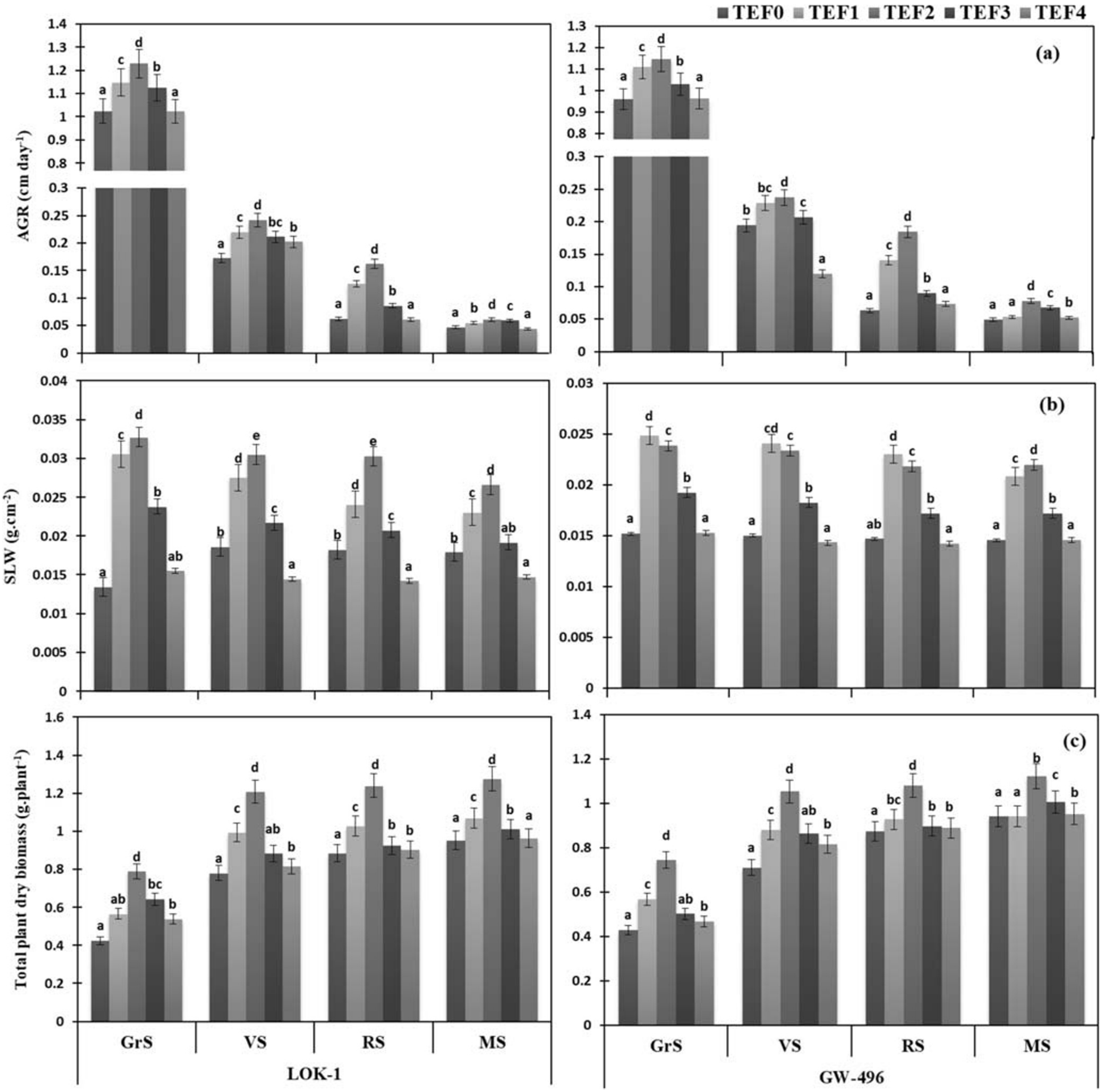


Fig. 1 RGR (**a**), specific leaf weight (**b**) and plant biomass (**c**) of *Triticum aestivum* L. (cv. LOK-1 and GW-496) grown under textile effluent fertilization (TEF) at germination (GrS), vegetative (VS), reproductive (RS) and maturity stage (MS). Letters around the bars indicate statistically significant differences among each treatments ($\alpha = 0.05$) by TMRT

both cultivars. Chlorophyll content may decrease by imbalance in soil nutrient such as excess of heavy metals, limited dose of minerals and micronutrient reallocation via phloem, resulting into slow rate of photosynthesis (Shah et al. 2017; Singh and Rathore 2019). In such cases, degradation of transitory starch helps to provide the energy and carbon source to the plant body and guard cells for survival (Santelia and Lunn 2017). At the vegetative growth stage, protein content of both wheat cultivars was significantly higher under all the textile effluent fertilization treatment compared with the TEF0, which is further increased at the reproductive stage (Fig. 2(c), Table 1). Increasing the chlorophyll and soluble protein content at lower concentration of effluent application agreed with Gupta and Mittal (2017) due to the addition of nutrients and salts. However, plants grown in high concentration of effluent have reduced chlorophyll synthesis and protein content simultaneously in the

Table 1 F-ratio and significance level of two-way ANOVA test for morphological (plant biomass, RGR, SLW) and biochemical (chlorophyll, protein, proline, transitory starch, soluble sugar and reducing sugar) characteristics of *Triticum aestivum* L. (cv. LOK-1 and GW-496) grown under textile effluent fertilization (TEF) at different growth stages

Dependent variables	Growth stages		TEF		Growth stages × TEF	
	LOK-1	GW-496	LOK-1	GW-496	LOK-1	GW-496
Total plant biomass	322.09^a	61.48^a	10.2^b	16.70^a	16.77^a	9.84^a
RGR	208.72^a	329.32^a	2.96^c	5.83^b	4.22^b	8.76^*
SLW	12.88^b	23.19^c	84.32^a	74.39^a	8.19^b	3.88^b
Total chl	68.92^a	146.07^a	9.76^b	21.22^a	11.08^a	36.16^a
Chl *a*/*b* ratio	316.01^a	95.29^a	113.16^a	81.64^a	3.87^b	8.26^a
Protein	20.09^b	103.02^a	67.81^a	119.68^a	3.16^b	2.88^b
Proline	9.19^b	8.99^b	14.67^a	20.16^a	18.67^a	8.55^b
Transitory starch	104.24^a	97.15^a	261.13^a	122.62^a	18.52^a	8.94^a
Soluble sugar	9.96^a	81.56^a	316.09^a	285.39^a	5.87^a	9.62^b
Reducing sugar	54.38^a	61.12^a	85.53^a	101.24^a	8.69^a	4.88^a

*Non-significant

$^a \leq 0.001$

$^b \leq 0.01$

$^c \leq 0.05$

present experiment. It may be due to the heavy metal interference with synthesis of amino levulinic acid and protochlorophyllide reductase in tetrapyrrole pathway (Ali et al. 2015).

Proline is considered a compatible osmolyte and its accumulation in response to different stresses has been reported in several plant species. Proline helps to stabilize protein through facilitating the folding (Liang et al. 2013). In the present study, proline content was found lowest under TEF1 and increasing significantly under textile effluent fertilization of both wheat cultivars (Fig. 2(d)). Among the cultivars, accumulation of proline was higher in LOK-1 than GW-496. It may attribute the plant strategy to overcome alkalinity-induced oxidative stress of textile effluent.

Effects of textile effluents on transitory starch and sugar content

Accumulation of transitory starch in leaves is an environment-dependent multifaceted process affected through stress caused by nutrient deficiency or excess of heavy metals in growing medium (Boussadia et al. 2010). Synthesis and degradation mechanisms of transitory starch consisted with several enzymatic activity either in dark or under stress conditions. Transitory starch typically accumulates in leaves gradually during the day and is degraded at night to support heterotrophic metabolism (Zanella et al. 2016). However, under stress condition including metals, heat and cold (Lu and Sharkey 2006; Rosa et al. 2009), starch is degraded to release sugar and sugar-derived osmolytes in the presence of light (Zanella et al. 2016; Thalmann and Santelia 2017). In our experiments, a highly significant increase in transitory starch was observed in both cultivars treated with low and moderate dose of textile effluent (Fig. 3(a)), whereas a slight decline in TEF4 was observed at all the growth stages. It may attribute the proper nutrient supply present in textile effluents while declined starch level at higher concentration could be explained as the excess accumulation of trace metals present in effluent as reported by Shukla et al. (2003). Lowest accumulation of transitory starch was found in TEF0 (Fig. 3(a)), because the absence of exogenous nutrients substantially reduced starch breakdown and maltose (reducing sugar) accumulation in chloroplast (Lu and Sharkey 2006) or it may result from the increase in photosynthetic rate in leaves (Lu and Sharkey 2006; Rosa et al. 2009).

Similar to transitory starch, improvement of foliar soluble sugar was also observed as a result of textile effluent application in both cultivars than their respective control, while under TEF4 fertilization, it was declined significantly at all the growth stages of plant (Fig. 3(b)). Similar to this study, Gupta and Mittal (2017) explained increased soluble sugar accumulation at lower concentration of textile effluent in *Tagetes erecta* due to micronutrient availability at lower concentration.

Contrary to transitory starch, concentration of reducing sugar of wheat cultivars was declined by textile effluent fertilization. Minimum reducing sugar was found in TEF1 that progressively increased with increasing textile effluent concentration as fertilizer (Fig. 3(c)). Shukla et al. (2003) reported declined level of reducing sugar in *Triticum aestivum* under increasing concentration of Cd. Maximum content of reducing sugar was found in TEF0 at all growth stages in both wheat cultivars. Similar result was also observed in two olive cultivars by Boussadia et al. (2010) under nitrogen deficiency,

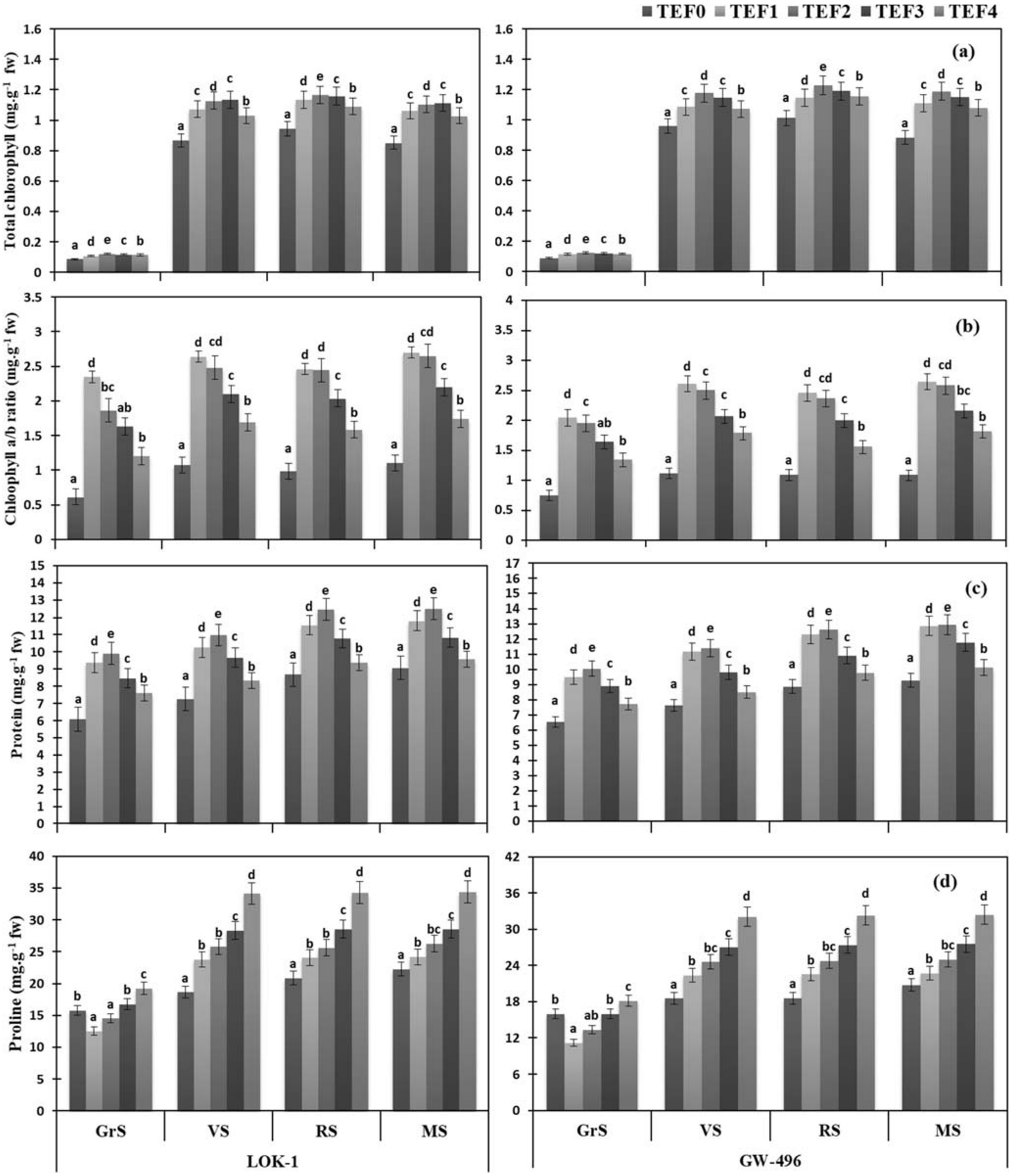


Fig. 2 Total chlorophyll (**a**), chlorophyll *a*/*b* ratio (**b**), protein (**c**) and proline accumulation (**d**) of *Triticum aestivum* L. (cv. LOK-1 and GW-496) grown under textile effluent fertilization (TEF) at germination (GrS),vegetative (VS), reproductive (RS) and maturity stage (MS). Letters around the bars indicate statistically significant differences among each treatments ($\alpha = 0.05$) by TMRT

while mechanisms behind gradual enhancing of reducing sugar at increasing level of effluent application can be explained as the functioning of higher alkaline nature of textile effluent or metal accumulation. As the plant gain maturity, reducing sugar level was found to be declined (Fig. 3(c)). Robertson et al. (1996) explained the reduction in the accumulation of

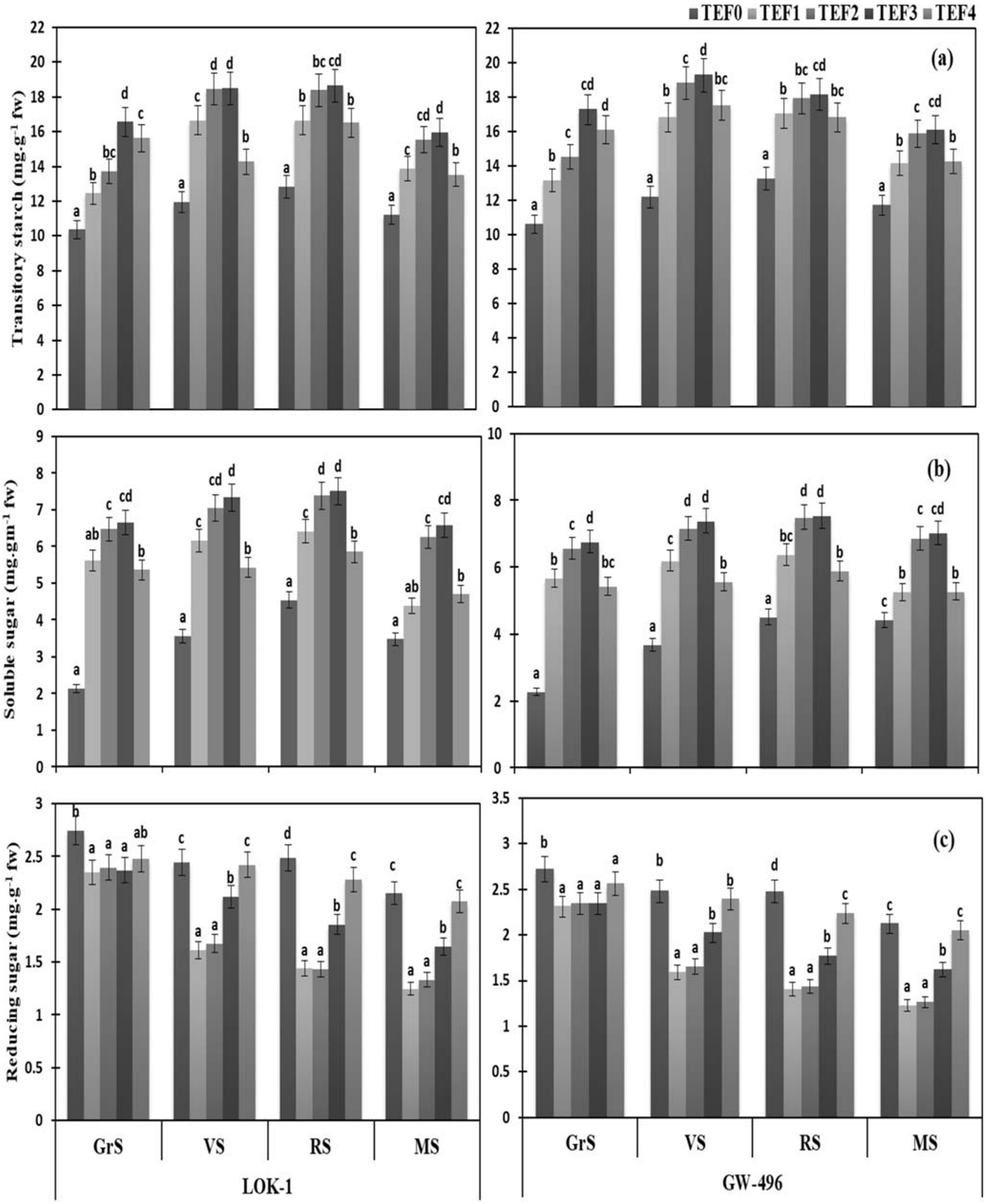


Fig. 3 Transitory starch (**a**), soluble sugar (**b**) and reducing sugar (**c**) of *Triticum aestivum* L. (cv. LOK-1 and GW-496) grown under textile effluent fertilization (TEF) at germination (GrS), vegetative (VS), reproductive (RS) and maturity (MS). Letters around the bars indicate statistically significant differences among each treatments ($\alpha = 0.05$) by TMRT

reducing sugars with crop age as reduction in actively growing leaves and branches after vegetative growth and mature leaves contain little reducing sugar. Statistically, the effect of textile effluent fertilization was highly significant for transitory starch, soluble sugar and reducing sugar accumulation in both wheat cultivars (Table 1).

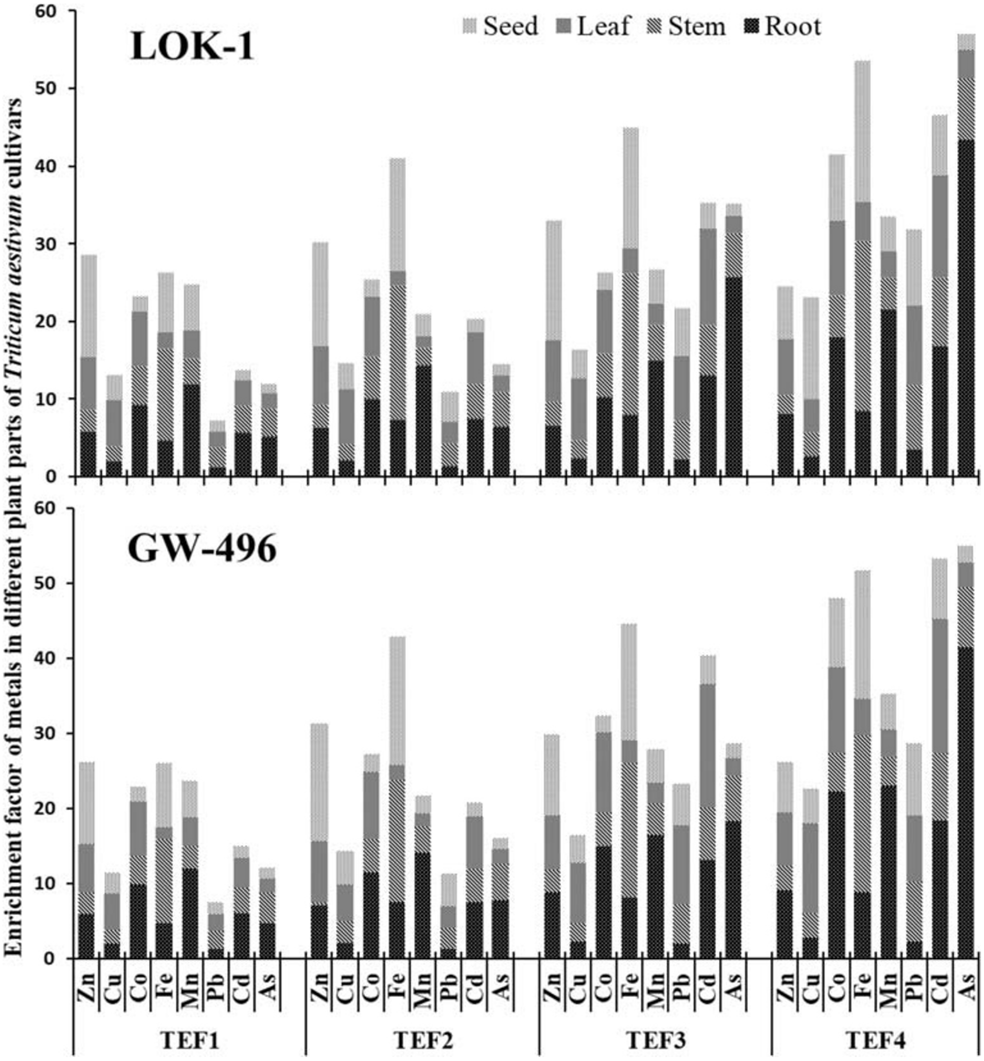
Metal accumulation, enrichment and transfer in plant tissues of wheat

Fe and Zn are higher in root of both experimental plants as the effluent concentration increases to TEF4 than TEF0. Concentration of Fe, Mn, Cu, Co and Zn increased significantly in root, stem, leaf and seed with decreasing the effluent dilution. Interestingly, no noticeable differences were found in the Pb and Co concentration in roots, stems, leaves and seeds of both cultivars (cv. LOK-1 and GW-496) among the treatments (Fig. S2 and S3). Cd and As concentrations in each treatment are significantly different (at $p < 0.05$), while the highest concentration was found in root of GW-496 at TEF4. The present results show that increasing transitory starch content increased the plant growth, and trace metal accumulation in leaf and seed in lower concentration of textile effluent shows higher heavy metal accumulation decline in the transitory starch at TEF 4. Zappala et al. (2014) reported that excess Cu disturbed the development of chloroplast and starch granules accumulated in leaves of *Prosopis pubescens*. However, low-dose Pb and Ni increased the starch content in leaves of bean (Nyitrai et al. 2004). Higuchi et al. (2015) reported in his study that accumulation of heavy metals (Cd) in leaf promoted the α-glucan concentration (one of the photosynthesis inhibitor), accounting the decrease in transitory starch accumulation. Analysed value of heavy metals in wheat plant parts and grains was below the permissible limit, and hence safe for consumption (Hassan et al. 2013).

Absorption and accumulation of trace metal in plant tissue depend upon many factors, which include temperature, moisture, pH and nutrient availability. Therefore, TF and EF were calculated to evaluate the transfer and enrichment of micronutrients from the plant to grains. Based on these results, we calculated the enrichment factor (Fig. 4). The sequential metal enrichment in plant tissues was Zn > Fe > Mn > Co > Cu > Cd > As>Pb. Wheat cultivar GW-496 accumulated higher concentration of trace element in tissues than the cultivar LOK-1. Grains of both wheat cultivars have increased enrichment factor value of trace elements at increasing effluent concentration, which indicated the moderate to significant enrichment in root, stem, leaf and grain. Results from the present study of differences of metal accumulation (mobility) from root to grain are consistent with the results by Bose and Bhattacharyya (2008), in which Pb, Zn and Cd concentrations were higher in roots and lower in the shoots and grains. The symplastic transport via the phloem allows redistribution of nutrients, assimilates and pollutants within the plants (Page and Feller 2015). Trace element accumulation in the tissue of different plants resulted in a decrease of the biomass and the chlorophyll concentration in the leaves (Manios et al. 2003). The significant enrichment factor ($5 < EF < 20$) in the edible parts is an important criterion to assess the quality and suitability of crop species (Singh et al. 2010). Increasing enrichment factor of Zn > Mn > Fe in leaf, stem and grain indicates the possible ability of *Triticum aestivum* for trace metal uptake and nutrient availability in grains under lower range of textile effluent application (Singh et al. 2010). Enrichment factors in all the plant tissues of LOK-1 were found higher than GW-496 (Fig. 4). The result from the present study showed the higher enrichment factor of Zn followed by Fe, Mn, Co and Pb in different plant parts as the concentration increases (TEF 4). It may explain that the phloem mobility of heavy metals varies in a wide range. Zn is also mobile in plants and can be transported via the phloem to growing plant tissues (Page and Feller 2015). Enrichment value of Cd and As (pollutants and not a nutrient for plants) was found higher in plant tissues (in root) while higher concentration of textile effluent application (TEF 4) attributed it is less xylem-mobile but it is also bio-accumulated and redistributed via phloem when source availability increased (Gupta et al. 2010). Increasing enrichment in edible portion of food crops is an important source of trace metals into the human food chain (Chiroma et al. 2014). Results of this study showed the appreciable accumulation of trace metals in wheat grains at lower concentration of textile effluent application in comparison with control.

TF value of Zn and Co was higher (> 1) in all the treatments than control for root-aerial part; however, TF value for root-seed is < 1. TF value for Mn was lowest among all the textile effluent fertilizer concentration (Table 2). Translocation process of metals form source to sink was found higher in GW-496 than LOK-1. Translocation of heavy metals in plant tissues and to the grain is one of the key components of increasing availability and fortification of trace metals (Boussen et al. 2013). Translocation factor (TF) is a parameter used to describe the transfer of trace elements, and the ratio '> 1' means higher accumulation of metals in plant tissues from source (root) to sink (grains) (Sperotto et al. 2014). TF value of Co > Fe > Cu > Zn was higher than Pb > Cd > As > Mn in all the treatments from root to aerial part of metal translocation; this value of the internal transport of metals from root to grains was restricted. This immobilization of metals in plant root cells is considered as an exclusion as well as mobility strategy in plant cells (Gupta et al. 2010). Lower TF value in control than textile effluent–treated plant was also observed in the present study, which attributes the variation in physiochemical properties of soil (Boussen et al. 2013). Variation in both cultivars' (GW-496 > LOK-1) response to the different metal

Fig. 4 Vertical rectangle evolution of Zn, Cu, Co, Fe, Mn, Pb, Cd and As enrichment factors in plant tissues (root, stem, leaf and seed) of *Triticum aestivum* L. cv. LOK-1 (**a**) and GW-496 (**b**) grown under different dilution of textile effluent fertilization (TEF)



uptake could be due to the mechanisms and genotypic variation among the cultivars (Singh et al. 2016). The result shows that the distribution of metals from root to aerial was higher while root to seed was lower at TEF 4. It might be due to the lack of translocatable carbohydrates within the phloem under stress condition generated through Cd, As and Pb at higher concentration of textile effluent (Lemoine et al. 2013).

Relative correlation of transitory starch with morphological and biochemical parameters

Although very few reports are available concerning the actual mechanism of transitory starch in response to plant physical and biochemical condition, correlation study with transitory starch activity is supported by many researchers (Lu and Sharkey 2006; Weise et al. 2011; Zanella et al. 2016).

The study evaluated the linear correlation of morphological-biochemical parameters with respect to transitory starch at the vegetative growth stage (VS). An initial correlation analysis of the entire data set (not shown for every growth stage) observed a significant positive correlation among different variables with transitory starch activity. The correlation was analysed using the data of experimental treatments (TEF0, TEF1, TEF2, TEF3 and TEF4) of both cultivars. Photosynthetic pigments are strongly correlated with transitory starch activity (Fig. 5). Transitory starch is confined to leaves and other photosynthesizing tissues of the plant and the synthesis increased with increasing photosynthetic rate (Weise et al. 2011; Kalaji et al. 2018). Linear positive correlation of AGR and plant biomass shows the highly dependable activity while moderate correlation of SLW was shown in LOK-1 with transitory starch at each treatment (Fig. 5). Result attributed to the continuous increase in biomass, AGR and development of plant due to carbon accumulating as starch in the light and remobilizing at night to support respiration and growth (Thalmann and Santelia 2017; Shukla et al. 2017); thus, increasing the starch accumulation during the day enhances plant biomass and growth rate significantly

Table 2 Translocation factor or mobilization of trace/heavy metals from root to aerial part and root to seeds of *Triticum aestivum* L. (cv. LOK-1 and GW-496) grown under textile effluent fertilization (TEF)

	LOK-1					GW-496				
	TEF0	TEF1	TEF2	TEF3	TEF4	TEF0	TEF1	TEF2	TEF3	TEF4
Root-aerial										
Zn	0.395	0.466	0.449	0.462	0.310	0.323	0.479	0.477	0.346	0.350
Cu	0.623	0.883	0.937	0.993	0.792	0.631	0.801	1.050	1.061	1.249
Fe	0.394	1.158	0.946	1.029	1.273	1.154	1.090	0.969	1.055	1.246
Mn	0.181	0.305	0.155	0.287	0.192	1.203	0.325	0.262	0.285	0.201
Co	0.218	1.326	1.352	1.384	0.828	0.288	1.402	1.431	1.175	0.886
Pb	0.306	0.583	0.659	0.773	0.744	0.318	0.609	0.670	0.981	1.120
Cd	0.984	0.432	0.439	0.380	0.397	1.032	0.457	0.472	0.742	0.632
As	0.712	0.427	0.401	0.376	0.428	0.675	0.475	0.298	0.353	0.402
Root-seed										
Zn	0.0384	0.0866	0.0819	0.090	0.0316	0.048	0.089	0.107	0.0599	0.0359
Cu	0.066	0.101	0.102	0.108	0.382	0.076	0.106	0.164	0.123	0.125
Fe	0.0338	0.0562	0.0667	0.0262	0.0655	0.0727	0.065	0.082	0.068	0.068
Mn	0.011	0.053	0.020	0.0305	0.0213	0.012	0.046	0.019	0.032	0.024
Co	0.031	0.0634	0.0638	0.0658	0.143	0.030	0.059	0.061	0.044	0.125
Pb	0.006	0.008	0.018	0.0176	0.0178	0.006	0.008	0.021	0.0171	0.0264
Cd	0.0186	0.0412	0.0043	0.0047	0.0085	0.0187	0.046	0.047	0.0055	0.0082
As	0.105	0.023	0.023	0.015	0.017	0.092	0.029	0.0183	0.0198	0.0188

(Thalmann and Santelia 2017). A weak correlation was observed of SWL and AGR with transitory starch in the case of GW-496. It can be explained as the effect of textile effluent on the activity of β-amylase, a key enzyme which participates in the metabolism and conversion of transitory starch (Orzechowski 2008) at night or under stress condition.

Soluble sugar and reducing sugar are the monomeric products of starch. There is an overall strong positive correlation between soluble and transitory starch. Correlation graph of reducing sugar shows very weak positive correlation, and tends to lower concentration with increasing transitory starch in both plant cultivars (Fig. 5). Both experimental cultivars showed moderate positive correlation of protein and proline with transitory starch activity (Fig. 5). The response of soluble and reducing sugar to transitory starch is due to the enzymes participated and mechanisms involved in photosynthesis and metabolisms of sugars (Taybi et al. 2017). Under stress condition or according to the plant carbon requirement, transitory starch is degraded into glucose and maltose (reducing sugar), and then exported to the cytosol to be used either for sucrose (soluble sugar) synthesis or as an energy source (Weise et al. 2011; Taybi et al. 2017).

Correlation of protein and proline with transitory starch is not well known. Jiang et al. (1999) studied and discussed in his study that the protein was directly correlated with photosynthetic activity and show strong correlation with each other in two rice cultivars, while soluble sugar and proline accumulation followed a similar trend under the salt stress of Quinoa seedling (Rosa et al. 2009). According to Claudia (2016), the degradation of transitory starch supported the biosynthesis of proline to develop osmotic stress response.

Principle component analysis

The characteristics of both wheat cultivars (LOK-1 and GW-496) and their correlation with slight difference in response against growth stage and textile effluent were analysed using PCA, shown in score and loading plots (Fig. 6). PC1 and PC2 accounted for 57.76 and 23.50% of the total variance, respectively. Score plots showed that increasing concentration of textile effluents increased the PC1 at vegetative and maturity growth stage while decreased the PC2, vice versa at higher concentration (Fig. 6(a)). Thus, lower concentration showed highest fertilization effects for all the evaluated plant characteristics. The AGR strongly responded at germination stage of the plant growth at lower concentration of textile effluent fertilization (Fig. 6(b)). The bi-plot of principle component analysis (PCA) provides a comprehensive result of the interrelationship between all studied variables. It explains response of parameters for evaluating the effects of textile effluent fertilization against the control at each growth stage of the plant. The correlation between variables was examined using the

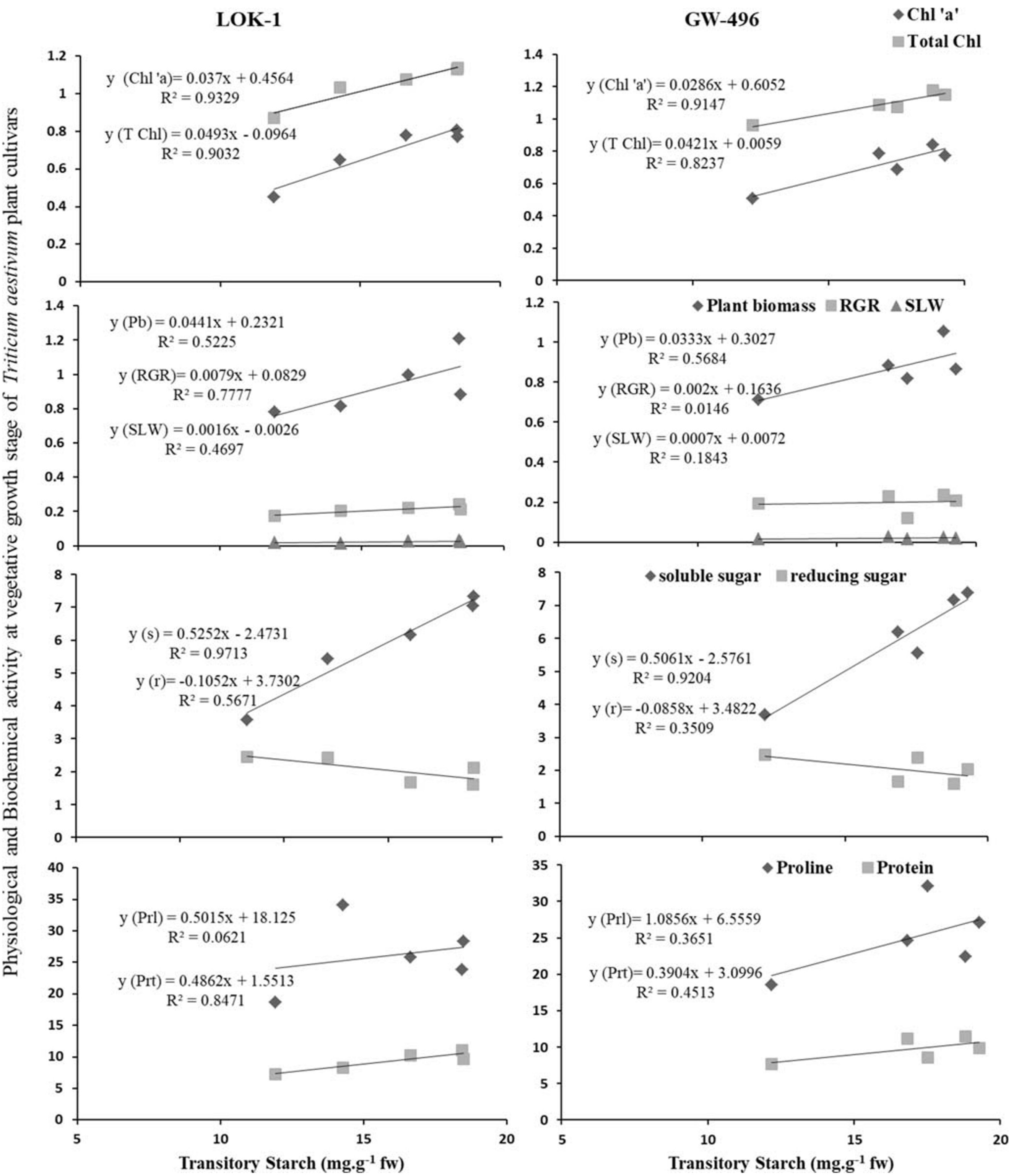


Fig. 5 Pearson correlation analysis between morphological (total plant biomass, RGR, SLW) and biochemical (Chl '*a*', total Chl, reducing sugar, soluble sugar, proline and protein) responses with transitory starch activity of *Triticum aestivum* L. (cv. LOK-1 and GW-496). Coefficient (R^2) value close to 1 expressed the strong positive correlation between the subsets

angles on bi-plot of PCA (El-Hendawy et al. 2017). Variables, i.e. transitory starch, soluble sugar, dry biomass, chlorophyll *a*, *b*, total chlorophyll and protein, were grouped together. This suggested that these parameters had a close relationship between them and are strongly active in vegetative and maturity growth stage at lower level of effluent fertilization,

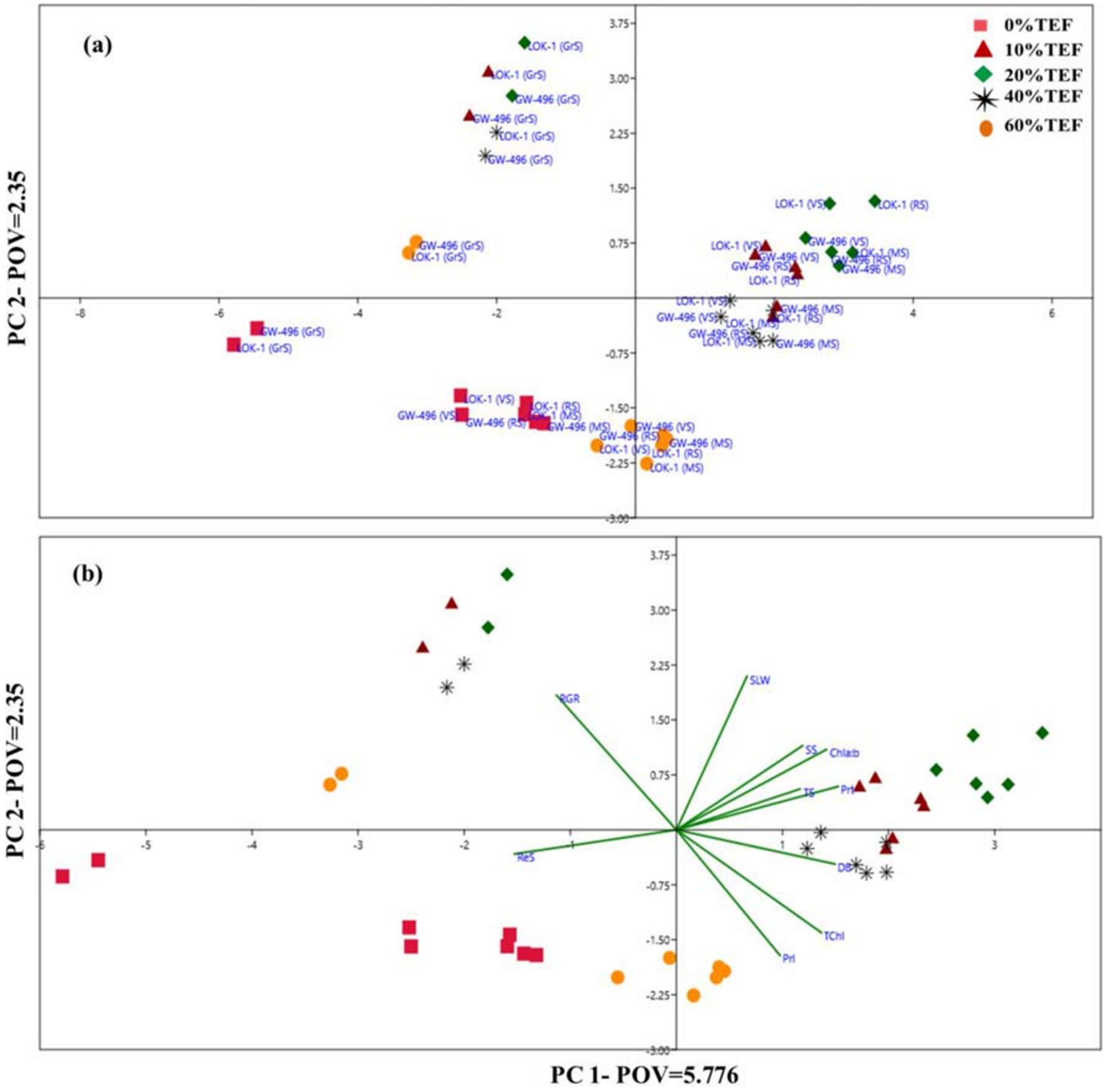


Fig. 6 Score (**a**) and loading plots (**b**) of principle component analysis (PC1 and PC2) of all the characteristics, growth stages, textile effluent concentration and both cultivars of *Triticum aestivum* L. (cv. LOK-1 and GW-496)

because lower concentration of textile effluent helps to promote growth to provide nutrients (Singh and Rathore 2018), while straight angle of SLW expressed its lower response at vegetative and maturity in lower effluent concentration. However, reducing sugar found active at control treatment (0% TEF), and straight angles of proline expressed its activity at higher concentration (60% TEF) of textile effluents (Fig. 6(b)), which attributed the osmotic response of plant against generated stress at higher concentration in both cultivars.

Relative correlated efficiency of transitory starch with accumulated trace elements in leaves

The study evaluated the linear positive correlation of transitory starch with respect to different trace element (Zn, Cu, Co, Fe, Mn, Pb, Cd and As) accumulation in leaves of both cultivars (Figs. 7 and 8). Correlation coefficient for Zn, Cu, Mn and Fe accumulation showed lower correlation with transitory starch (Fig. 7(a), (b), (c) and (d)) varying the R^2 value, i.e. 0.3824, 0.4881, 0.3878 and 0.3165, respectively, in LOK-1, while moderate relationship was found with trace element (Zn, Cu, Mn and Fe) accumulation and transitory starch in the case of GW-496. Correlation coefficient represents the moderate relationship of Co ($R^2 = 0.6617$ and 0.7823) while a strong relationship of Pb ($R^2 = 0.7678$ and 0.7996) with transitory starch in leaves of cv. LOK-1 and GW-496 (Fig. 8(a) and (b)) respectively. Weak correlation coefficient of trace metals and transitory starch might explain the indirect role of trace elements (Zn, Mn, Cu and Fe) to activate some beneficial enzymes or photosynthetic activity,

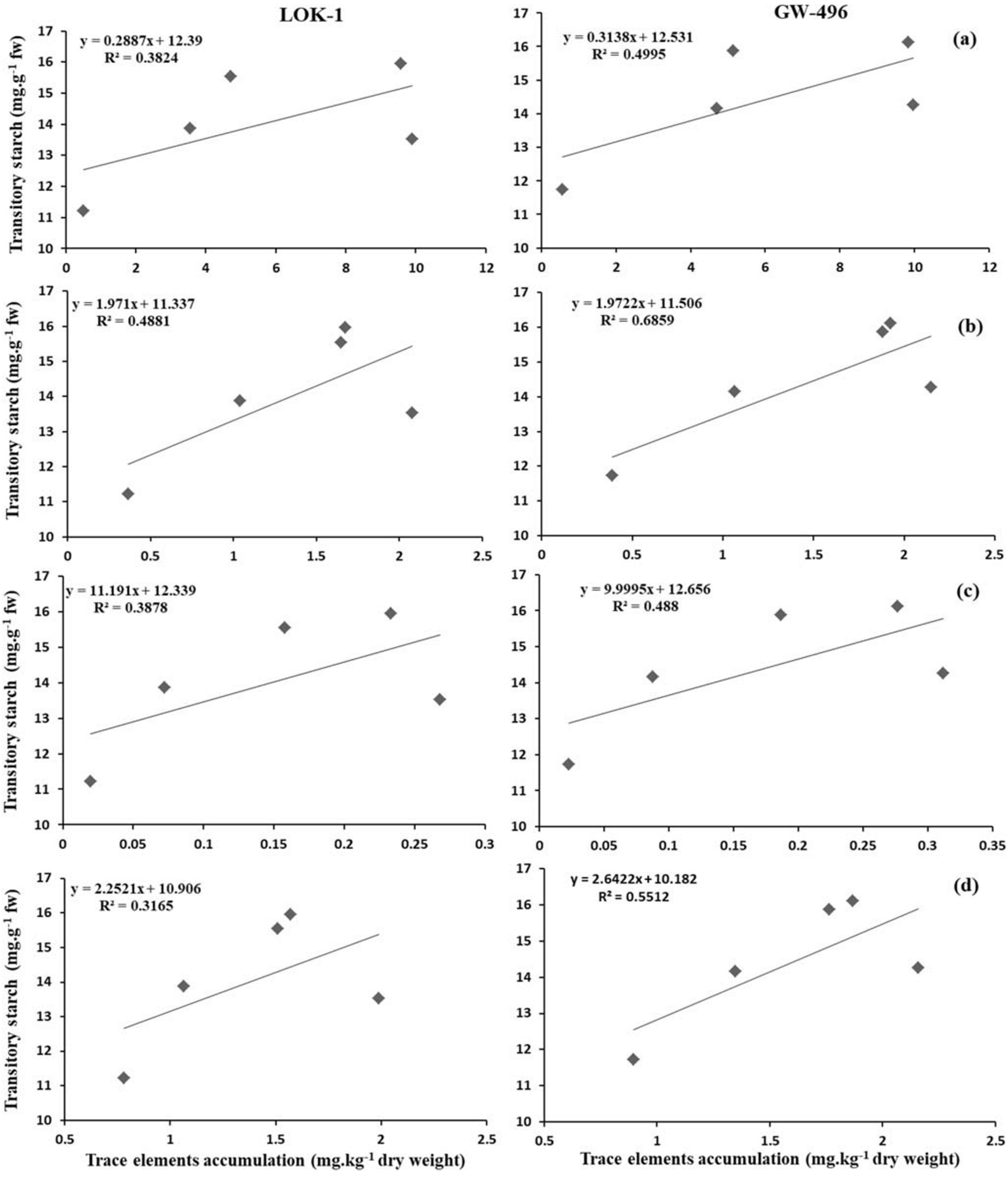


Fig. 7 Pearson correlation analysis between transitory starch activity at maturity of plant growth in response to Zn (**a**), Cu (**b**), Mn (**c**) and Fe (**d**) in leaf of *Triticum aestivum* L. (cv. LOK-1 and GW-496). Coefficient (R^2) value close to 1 expressed the strong positive correlation between the subsets

while toxic effects of higher concentration in leaves via anatomical changes, antagonistic effects or disturbed enzymatic activity (Singh et al. 2016). Sridhar et al. (2005) reported decreased starch content in leaves of *Brassica juncea* treated

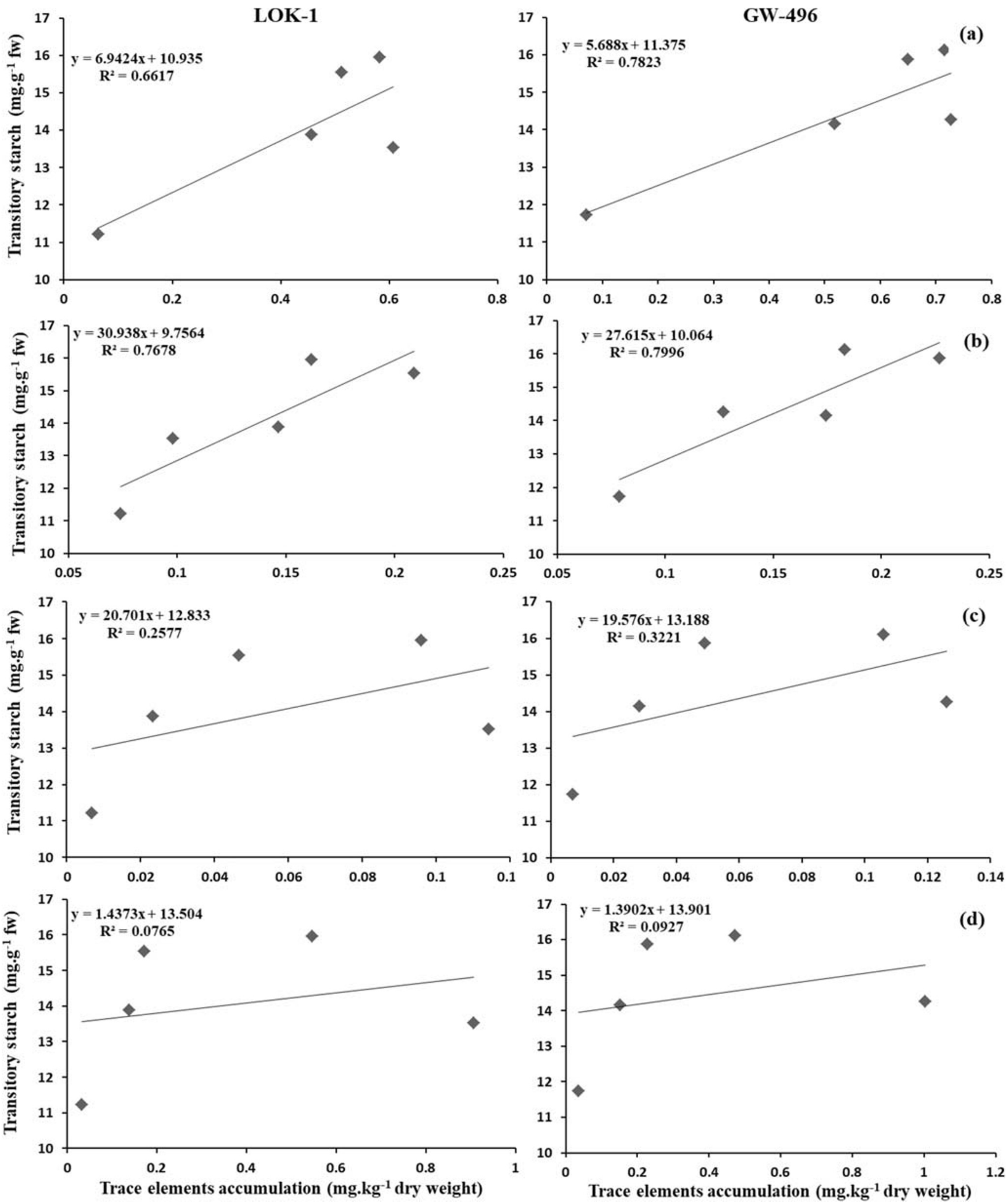


Fig. 8 Pearson correlation analysis between transitory starch activity at the maturity stage of plant growth in response to Co (**a**), Pb (**b**), Cd (**c**) and AS (**d**) in leaf of *Triticum aestivum* L. (cv. LOK- and GW-496). Coefficient (R^2) value close to 1 expressed the strong positive correlation in between the subsets

with high concentration of Zn. Strong correlation of Co with transitory starch in leaves might explain the requirement of plant sufficient levels of cobalt in the system in order to properly expand the leaf discs (El-Metwally and El-Saidy 2016) and increase the photosynthetic pigments at lower concentration (El-Sheekh et al. 2003). A weak correlation was observed between Cd and As (Fig. 8(c) and (d)) accumulation with transitory starch in both cultivars explains its toxic effect in leaf biochemical activity.

Conclusion

Activity of transitory starch deals with plant growth by providing carbon as an energy source in the form of soluble sugar. Pearson correlation coefficient of morphological and biochemical parameters of plant with transitory starch confirms its role in growth and developments of plants. Study proves fertilization with 20% of textile effluents with 80% ground water could significantly increase transitory starch accumulation that plays a crucial role to increase the plant growth and specific leaf weight (SLW) which may account to facilitate the trace metal (Zn, Fe, Mn, Cu) accumulation and avoid the toxic metals in seeds of *Triticum aestivum*. Tolerance for morphological, biochemical and metal accumulation activity of cv. GW-496 was higher than the cv. LOK-1 in response to effluent application. The study concluded that the transitory starch plays a crucial role for plants under stress condition. Furthermore, the study also concluded that the fertilization of lower dose of textile effluent significantly improves the mineral status and production of wheat crop. Therefore, the fertilization with lower concentration of textile effluent for wheat crop may be a sustainable and economical way for nutrient cycle and disposal of industrial waste water.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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