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Impact of Sewage Effluent Amended with the Chlorophyte *Scenedesmus Quadricauda* or the *Cyanophyt Nostoc* sp. on Some Metabolic Pools of Wheat and Alfalfa Plants

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ABSTRACT

A pot experiment was conducted to study the influence of various irrigation regimes on some metabolic pools of wheat and alfalfa plants. The photosynthetic pigments and soluble proteins of the tested plants significantly increased when irrigated with sewage amended with Scenedesmus quadricauda or Nostoc sp. On the other hand, soluble carbohydrates decreased in wheat shoots at different treatments except those irrigated with sewage enriched with S. quadricauda. Sewage and that amended with Nostocsp caused promotion of total carbohydrates. Wheat plants with sewage water amended with Nostocspor with mixture of tap water and sewage have an inhibitory effect on free amino acids while alfalfa plants with sewage only enhanced them. Sewage effluent or that reclaimed with S. quadricauda caused significant increase in total lipids content of wheat shoots. In contrast, alfalfa plants exhibited significant decrease at all treatments. This study aimed to determine the effects of amended sewage effluent on metabolic pools of these plants. Estimated pools included the photosynthetic pigments, proteins (soluble and total), carbohydrate (soluble and total), free amino acids and total lipids in shoots of experimental plants.

Keywords: Sewage effluent, Afalfa, Wheat, Algae, Carbohydrates, Proteins, Amino acids

INTRODUCTION

Water is an essential resource but a severely limited one in most Mediterranean countries [1]. Therefore, there is an urgent need to conserve the fresh water and to use the lower quality water in irrigation [2]. The reuse of treated municipal wastewater in such countries is less expensive and considered an attractive source of irrigation. Sewage water and sewage sludge are being used to improve soil properties and fertility in order to increase production plant [3]. Sewage water is a complex mixture in terms of chemical, biological, and physical characteristics. It is part of the most renewable wastewaters, while it should not be, at least in arid and semiarid regions [4]. The biomass of plants may share in solving the predicted energy crisis and using wastewater in agriculture might play a role in. In this respect, a linear increase in most nutrients such as plant nitrogen as a result of sludge amendment was reported [5].

Algae, performing oxygenic photosynthesis, are historically known to play a remarkable role in the treatment of municipal wastewater, as they have fundamental role in oxygen/carbon dioxide recycling with a net sequestration of CO_2 (a greenhouse gas). Algae have also a potential for nitrogen and phosphorous uptake into the cells; thus acting as fertilizers by mineral recycling [6]. Numerous authors revealed that application of algal extracts have beneficial effects on seed germination and establishment, improved crop performance, yield and elevated resistance to biotic and abiotic stress [7,8].

Vessey [9] defined a bio-fertilizer as a substance that contains living organisms that when applied to seed, plant surface, or soil, colonize the rhizosphere of plants and promote growth by increasing supply or availability of primary nutrients to the host plant. Microbial inoculation or bio-fertilizer application is an important component of organic farming as the microbes help to solubilize and mobilize elements to plants, produce plant growth promoting hormones, vitamins and amino acids and control plant pathogenic fungi also it improves soil health and increases crop production [10]. In this respect, the stimulatory influence of some organic and bio-fertilizers (sewage effluent, yeast, *Nostoc* and diatomaceous earth) on growth, yield, metabolic pools and seed oils of *Nigella sativa* L. grown in calcareous soil has been reported [11].

Improvement of soil fertility using sewage sludge has been reported widely in many recent studies [12-14]. The beneficial impact of sewage sludge applications is important, especially in soils that are poor in organic matter content such as those of the Arabian Peninsula with high temperatures during the summer, which promote the high annual mineralization of organic matter [15].

Alfalfa (*Medicago sativa* L.) is an important rotational crop, providing soil structure, nitrogen input, and pest management benefits with highly valued animal feed because of its high protein content and high intake potential [16]. Also, wheat (*Triticum aestivum* L.) is an edible, most important grain and one of the oldest cereal crops; it grows under a wide range of climates and soils but best adapted to temperate regions [17]. In a previous work, improving growth of alfalfa and wheat plants cultivated in sand soil of Taif area and irrigated with sewage effluent reclaimed *S. quadricauda* or with the Nostoc sp. has been published elsewhere [6]. This study aimed to trace the various alterations in metabolic pools of these plants. Estimated pools included the photosynthetic pigments, proteins (soluble and total), carbohydrate (soluble and total), free amino acids and total lipids in shoots of plants mentioned above.

MATERIAL AND METHODS

Purification, Identification and Maintenance of Cyanobacteria and Algae from Sewage Effluent

The cultivation and isolation of the cyanobacteria and algae that might be persisting as resting stages is carried out using the moist plate method [18]. Five replicate Petri-dishes (9 cm diameter) were inoculated, each with 1 ml of waste water and 20 ml of the molten medium. For blue green algae, Petri-dishes were incubated at 30 ± 1°C and16/8 hr. light/dark cycle with a white light intensity of 3000-4000 Lux; colonies are counted after about 25 days of incubation. Purified colonies or filaments of cyanobacteria and algae were picked and transferred to plates of BG-12 agar [19]. Cyanobacterial and algal species are examined by means of a binocular microscope and identified as earlier [20,21]. Algae and cyanobacteria, which were isolated from wastewater collected from Taif sewage water treatment plant were inoculated to sewage effluent, let grow and enrich for 24 h.

Plant Material

A pot experiment was conducted at Biology Department, Faculty of Science, Taif University, Saudi Arabia during 2015/2016 to investigate the effect of sewage effluent on some metabolic pools of wheat and alfalfa plants. Grains of wheat and seeds of alfalfa were sown directly in plastic pots (15 cm diameter) containing sand soil from Taif Governorate and irrigated with the treatments below.

Irrigation Regime

The experimental pots of wheat and alfalfa plants were irrigated up to the field capacity twice a week with the following irrigation regimes:

•Tap water, control (TWC)

•Sewage effluent (SE 100%)

•Sewage effluent mixed with tap water at a ratio of 1/1 (STW)

•Sewage effluent enriched with the green alga *Scenedesmus quadricauda* (XSE)

•Sewage effluent enriched with the blue green alga Nostoc sp. (YSE)

Analytical Methods

Assessment of photosynthetic pigment fractions

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were estimated using the spectrophotometric method [22] and presented as mg/g FW (fresh weight).

Assessment of soluble and total carbohydrates

The anthrone sulphuric acid method [23,24] was used to quantify soluble sugars and saccharides resulting from hydrolyzed polysaccharides; both fractions were calculated as mg/g DM (dry mass) using a calibration curve of glucose.

Assessment of soluble and total proteins

Soluble and total proteins were assessed according to the Lowry method [25] and the data were expressed as mg /g dry mass (DM) using a calibration curve of egg albumin.

Assessment of free amino acids

Free amino acids were assessed spectrophotometrically [26] and their concentration was calculated as mg/g DM using a calibration curve constructed using glycine.

Assessment of total lipids

Assessment of lipid contents in plant tissues was carried out as described [27] taking into consideration the modifications suggested [28].

Statistical Analysis

All data obtained were expressed as mean of three replicates ± standard deviation (SD). For each investigated parameter, one-way analysis of variance (ANOVA), using the (SPSS version 19) statistic package was applied. For comparison of the means, the Duncan's multiple range tests (p> 0.05) were utilized.

RESULTS AND DISCUSSION

Photosynthetic Pigments

Chlorophyll a (Chl. A) contents of wheat plants were significantly enhanced, those of chlorophyll b (Chl. B) were significantly lowered while carotenoids were relatively increased by the various treatments applied. On the other side, irrigation with sewage effluent enriched with the green alga *Scenedesmus quadricuda* (XSE) highly promoted pigment fractions in alfalfa plants, followed by sewage effluent/tap water mixture (STW 1/1) while sewage effluent enriched with the cyanophyte *Nostoc* (YTE) exerted no effect relative to other treatments (Figure1A and Figure 1B).

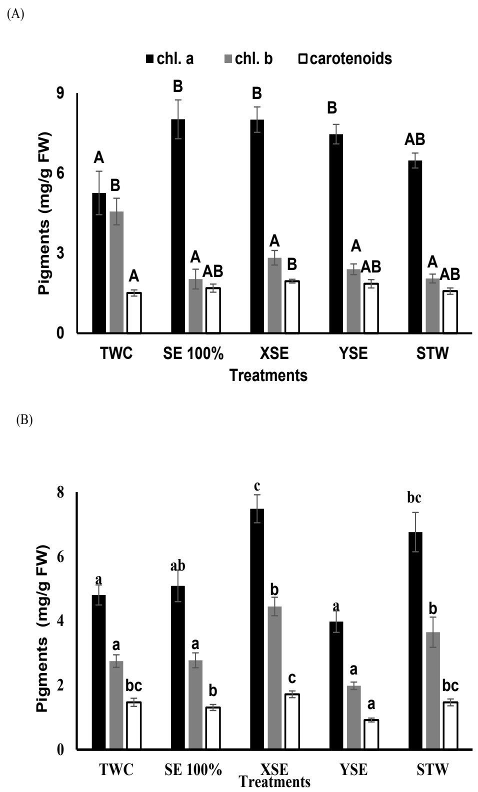


Figure 1: Photosynthetic pigments of wheat and alfalfa plants as affected by different treatments. TWC, (tap water), SE 100 % (100 % sewage effluent), XSE, (Sewage effluent enriched with the green alga *Scenedesmus quadricauda*), YSE (Sewage effluent enriched with *Nostoc* sp.), STW (mixture of tap water with sewage effluent in a ratio of 1/1). The results are means of five replicates (± SE). Different letters are significantly different at $P<0.05$

Soluble and Total Carbohydrates

Soluble carbohydrate contents of wheat plants exhibited non-significant effect when irrigated with all treatments; except YSE that is exerted significant inhibition. Alfalfa, however, showed lower contents of soluble carbohydrates in their shoots than in those of wheat plants, regardless of the treatment applied. Alfalfa irrigated with sewage effluent enriched with *S. quadricauda* or Nostoc sp. followed by sewage/tap mixture (XSE, YSE or STW) exhibited significantly increased soluble carbohydrates content (Figure 2A). Total carbohydrate contents in shoots of the tested plants are shown in figure (Figure 2B). In wheat, they were reduced significantly with the application of various treatments, while alfalfa irrigated with sewage only (SE 100 %) or sewage amended with Nostoc sp (YSE) have positive effects in total carbohydrate contents compared with control (TWC) plants.

Free amino acids

The data presented in Figure 3 show that wheat treated with Nostoc-amended sewage water (YSE) or a mixture of tap water with sewage effluent (STW) exhibited a significant inhibitory impact on free amino acids compared with control plants irrigated with tap water. However, alfalfa plants, which were irrigated with sewage effluent only (SE 100%), recorded the highest significant values of amino acids but alfalfa irrigated with diluted sewage (STW) reduced the free amino acid contents.

Proteins (soluble and total)

The data presented in figure 4A indicated significant enhancement of soluble protein contents only in wheat plants irrigated with SE or YSE whereas application of XSE or STW significantly reduced this component. However, alfalfa plants (Figure 4A) exhibited significantly increased soluble protein pool at all treatments (except at XSE), compared with control plants (TWC). Total proteins, on the other side, were significantly promoted at both plants (wheat and alfalfa) at all the treatments, exception-less, highest at STW (Figure 4B).

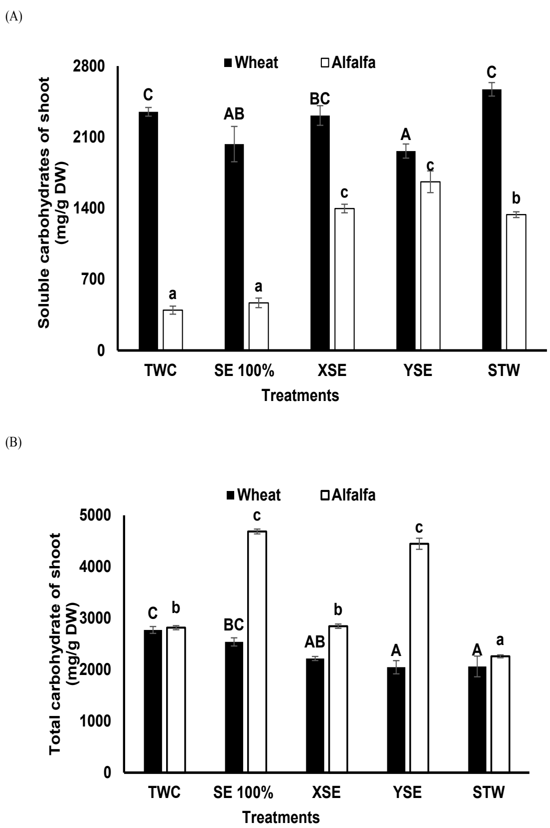


Figure 2: Soluble protein (A), Total protein (B) of wheat and alfalfa plants as affected by different treatments. TWC, (tap water), SE 100 % (100 % sewage effluent), XSE, (Sewage effluent enriched with the green alga *Scenedesmus quadricauda*), YSE (Sewage effluent enriched with *Nostoc* sp.), STW (mixture of tap water with sewage effluent in a ratio of 1/1). The results are means of five replicates (±SE). Different letters are significantly different at $P<0.05$

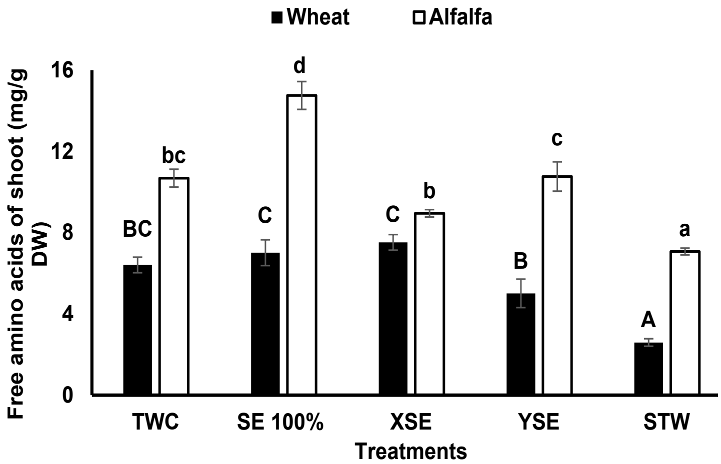
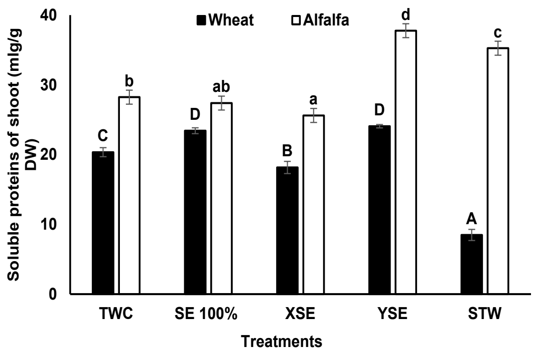


Figure 3: Free amino acids of wheat and alfalfa shoots as affected by different treatments. TWC, (tap water), SE 100 % (100 % sewage effluent), XSE, (Sewage effluent enriched with the green alga *Scenedesmus quadricauda*), YSE (Sewage effluent enriched with *Nostoc* sp.), STW (mixture of tap water with sewage effluent in a ratio of 1/1). The results are means of five replicates (± SE). Different letters are significantly different at $P<0.05$

(A)



(B)

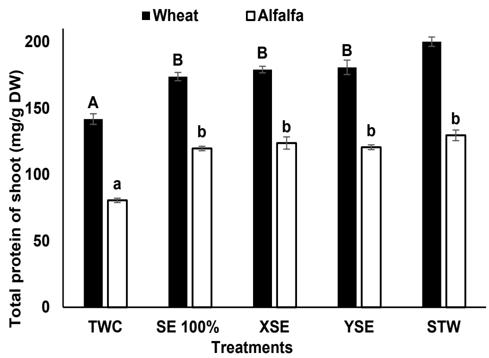


Figure 4: Soluble protein (A), Total protein (B) of wheat and alfalfa plants as affected by different treatments. TWC, (tap water), SE 100 % (100 % sewage effluent), XSE, (Sewage effluent enriched with the green alga *Scenedesmus quadricauda*), YSE (Sewage effluent enriched with *Nostoc* sp.), STW (mixture of tap water with sewage effluent in a ratio of 1/1). The results are means of five replicates (±SE). Different letters are significantly different at $P<0.05$

Total lipids content

The biochemical characteristics are used more infrequently, though they determine the genotype plasticity and are the basis of physiological processes, many of which occur with membranes. In this investigation, sewage effluent only (SE 100%) or that reclaimed with *S. quadricauda* (XSE) has noticeable and significant enhancing effect on total lipids content of wheat plants when compared with the control ones. In contrast, alfalfa plants exhibited a significant reduction of total lipids at all treatments (Figure 5).

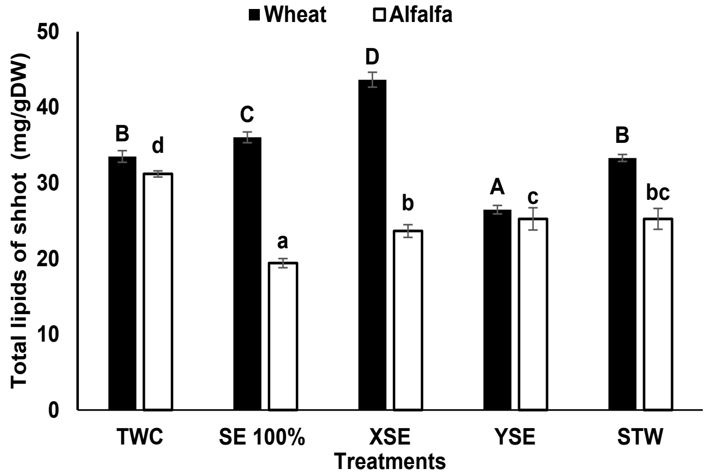


Figure 5: Total lipids of wheat and alfalfa shoots as affected by different treatments. TWC, (tap water), SE 100 % (100 % sewage effluent), XSE, (Sewage effluent enriched with the green alga *Scenedesmus quadricauda*), YSE (Sewage effluent enriched with *Nostoc* sp.), STW (mixture of tap water with sewage effluent in a ratio of 1/1). The results are means of five replicates (±S E). Different letters are significantly different at $P < 0.05$

DISCUSSION

Chl. a contents of wheat plants were significantly enhanced, those of Chl. b were significantly lowered while carotenoids were relatively increased by most of the various treatments applied. These results are in harmony with the enhancement of total chlorophyll contents in both wheat and alfalfa plants by algae-amended sewage effluent compared with sewage without amendment [6]. Besides, Subramaniyan et al [29] found that chlorophyll a content was maximal in *Oscillatoria annae* with *Lantana camara* 500 ppm, whereas chlorophyll b increased in *Oscillatoria annae* with *Prosopis juliflora* 250 ppm. Seaweed fertilizers were effective in increasing growth and yield of maize and tomato plants [29-31]. The increase of chlorophyll content was attributed to increased photosynthetic electron transport [32], stimulation of pigment biosynthesis [33] and interaction with the thylakoid membrane surface [34].

Soluble and total carbohydrate contents were variably altered depending on the treatment applied. Our results are in agreement with those of Rajula and Padmadevi [35] who emphasized the increases in biochemical contents like proteins, carbohydrates, amino acids in the seedling of *Helianthus annuus* L. grown in effluent blended with cyanobacteria. In accordance with these results, [36] stated that nitrogen and carbohydrate contents play a vital role in regulating seedling growth through reserve mobilization and photosynthetic gene expression.

Free amino acids play a vital role in plant metabolism as they connect carbon assimilation with nitrogen metabolism [37]. The results of this investigation show that wheat plants treated with *Nostoc*-amended sewage water or a mixture of tap water with sewage effluent (STE) significantly inhibited free amino acids contents compared with control plants irrigated with tap water. However, alfalfa plants, which were irrigated with sewage effluent (SE 100%), only recorded the highest significant values of amino acids but alfalfa irrigated with diluted sewage reduced the free amino acid contents. The accumulation of free amino acids in plants may be considered a stress marker; it may result from up-regulated biosynthesis or most probably from inhibited polymerization into proteins. In context with nitrogen metabolism, irrigation of *Solanum nigrum* with wastewater resulted in a significant accumulation of total free amino acids in root, shoots and leaves [37]. Also, these results are in accordance with those obtained previously [38] who reported that wastewater irrigation enhanced the accumulation of total free amino acids and proline contents in maize. [39] stated that the accumulation of free amino acids in plants under stress have important roles in metal toxicity tolerance via detoxifying heavy metals, regulating ion transport, regulating intracellular pH, ion transport, modifying stomatal conductance, and scavenging the ROS. Plants evolved some enzymes that convert amino acids, amides, and keto-acids and used them as carbon source to cope with stress when carbon deficiency becomes a limiting factor for growth and development [40].

The data indicated significant enhancement of soluble protein contents only in wheat plants irrigated with SE or YSE whereas application of XSE or STW significantly reduced this component. However, alfalfa plants exhibited significantly increased soluble protein pool at all treatments (except at XSE), compared with control plants (TWC). Total proteins, on the other side, were significantly promoted at both plants (wheat and alfalfa) at all the treatments, exception-less, highest at STW. These data are in agreement with those obtained by Mohiuddin et al. [41] who detected an increase in protein contents of wheat plants treated with different biofertilizers (Phosphert, Azofert, Biplin and Vitormone). Also, protein contents of *Lupinus termis* increased in seeds soaked in filtrate of *Cylindrospermum muscicola* algae [42] and postulated that the above mentioned changes in protein contents occurred according to either an increase or decrease in proteolytic enzymes that degrade the storage protein to soluble nitrogenous compounds, which may consequently utilized by the various parts of the *Lupinus termis* seedlings. In this respect, the filtrate of the cyanobacterium *Nostoc muscorum* increased the germination of wheat seeds significantly as well as their growth parameters and nitrogen compounds, compared with controls [43]. Also, the application of an extract from algae to soil or foliage increased ash, protein and carbohydrate contents of potatoes (*Solanum tuberosum*) [44].

The data in the literature indicate that plasticity of plants is a combination of morphological, biochemical, and physiological characteristics [45]. The biochemical characteristics are used more infrequently, though they determine the genotype plasticity and are the basis of physiological processes, many of which occur with membranes. Basic building blocks of membranes are lipid molecules [46]. Lipids are among the most prominent constituents of cell membranes, which play a fundamental role in cell permeability [47]. In this investigation, sewage effluent only (SE 100%) or that reclaimed with *S. quadricauda* (XSE) has noticeable and significant enhancing effect on total lipids content of wheat plants when compared with the control ones. In contrast, alfalfa plants exhibited a significant reduction of total lipids at all treatments. Various types of environmental stress, cause alterations in the physical properties of the membrane lipids in living cells. It seems likely that cells perceive these alterations via sensory proteins embedded in their membranes [48,49]. Also, these conditions enhance ROS formation, which has the potential to interact with many cellular components, resulting in inter alia damage to membrane lipids and proteins. Many reports indicate that oxidative stress is an important component of the plant response to various toxic chemicals such as heavy metals [50].

CONCLUSION

This study aimed to trace the various alterations in metabolic pools of studied plants. The results that photosynthetic pigments and soluble proteins of the tested plants significantly increased when irrigated with sewage amended with *Scenedesmus quadricauda* or *Nostoc* sp. Otherwise, soluble carbohydrates decreased in wheat shoots with different treatments except those irrigated with sewage enriched with *S. quadricauda*. Sewage and those amended with Nostoc sp caused promotion of total carbohydrate. Wheat plants with sewage water amended with Nostoc sp and mixture of tap water with sewage have an inhibitory effect on free amino acids, while alfalfa plants which effluent with sewage only enhanced them. Effluent with sewage only or those reclaimed with *S. quadricauda* caused significant increasing in total lipids content of wheat shoots. In contrast alfalfa plants exhibited significant decreasing with all treatments.

AUTHORS' CONTRIBUTIONS

All authors participated equally at all stages and parts of the paper.

No conflict of interest among the authors.

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