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Full Length Research Paper

Effect of industrial wastewater on total protein and the peroxidase activity in plants

Cüneyt Aki[1]*, Esra Güneysu[2] and Okan Acar[1]

[1]Department of Biology, Faculty of Arts and Science, Canakkale Onsekiz Mart University, Canakkale, Turkey.
[2]Antalya Ismet Inonu Vocational High School, Ministry of National Education, Antalya, Turkey.

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The aim of this study is to investigate the effects of industrial wastewaters on protein and the peroxidase activity in *Lycopersicon esculentum* Mill., *Capsicum annuum* L., *Phaseolus vulgaris* L. and *Vicia faba* L. Industrial wastewaters were taken from Dardanel Fisheries Company, Tekel alcoholic drinks companies' wastewater treatment plants and from one station which is located in the middle of the Sarıçay River. Wastewaters were applied to 6 weeks old plants with directly irrigation water. Physiological changes in the plants were observed by the means of measuring the protein and enzyme activity. The largest increase in protein was observed as 190.9 and 136.3% in *V. faba* treated with Sarıçay River water and Tekel wastewater, respectively. In *P. vulgaris* which was treated with Dardanel wastewater, the total protein amount increased by 84% compared to control plants. After the wastewater treatment, the peroxidase activity decreased in all plants. The largest peroxidase decrease was 80% in *L. esculentum* treated with Tekel wastewater. In *P. vulgaris*, peroxidase decreased by 59 and 51% when treated with Dardanel wastewater and Sarıçay River water, respectively. It was concluded that the increase in total protein amount and the decrease in peroxidase activity demonstrated the industrial wastewater's blocking effects on plants defense systems.

Key words: Wastewater, *Lycopersicon esculentum* Mill., *Capsicum annuum* L., *Phaseolus vulgaris* L., *Vicia faba* L., protein, peroxidase.

INTRODUCTION

Effluents of industrial processes and households contain hazardous genotoxic chemicals, some of which do not undergo degradation during wastewater treatment. As these chemicals very often have a lipophilic nature, they will end up in the wastewater sludge. Due to the growing application of wastewater sludge as a fertilizer on agricultural fields, there is a serious concern regarding the possibility that genotoxic chemicals can harm organisms in the ecosystems through their accumulation in the food chain. The aim of our research is to identify the physiological effects of different wastewater types on different plants by the means of measuring the total protein and the peroxidase activity. The cellular stress response is a ubiquitous defense mechanism activated when cells are confronted with stress. This stress which affects the plant's defense mechanism can be analyzed through the plant's defense response. In some cases wastewater treatment can influence biochemical pathways. The induction of the stress response leads to the expression of a group of proteins referred to as stress proteins, thought to protect the cell (Morange, 1997). In plants, stress genes are activated by developmental and environmental factors, hormonal stimuli and by microbial attack (Nover and Scharf, 1997). Chitinases, chitosanase and peroxidase are present in higher plants.

Peroxidases [EC 1.11.1.7] are the most important part of the multiple plant defense system and are mostly synthesized in the chloroplasts (Gabara et al., 2003). These enzymes are part of the multi-component defense system and are involved in defense reactions of plants against pathogens and every kind of stress factors. Peroxidases are involved in several physiological and biochemical processes such as cell growth and expansion (Lin and Kao, 1999), differentiation and development, auxin catabolism (Mansouri et al., 1999), lignifica-

*Corresponding author. E-mail: cuneytaki@comu.edu.tr. Tel: +902862180018/1606.

Table 1. The water analyses values of Dardanel Wastewater, Tekel Wastewater and Sarıçay River.

Parameter	Dardanel wastewater (mg/L)	Tekel wastewater (mg/L)	Sarıçay River (mg/L)
BOD	110	152	28*
COD	178	147	62*
Grease oil	32**	-	-
Total phosphate	17**	6	0.22*

*Higher values according to Water Pollution Control Standards, Table 1.
**Higher values according to Water Pollution Control Standards, Table 2.

tion (Sitbon et al., 1999), as well as abiotic and biotic stress responses (Medina et al.,1999). Peroxidases can form a new cell wall through biosynthesis. In the large family of peroxidases, the process of detoxification of H_2O_2 is particularly important (Ranieri et al., 2000). In the cell wall, PODs are present in soluble forms. The cell wall stiffening has been attributed mainly to peroxidases whose activity can be detected using the enzymatic assay mixture (Herbette et al., 2003). The aim of our research is to identify the physiological effects of different wastewater types on different plants by means of measuring the total protein and the peroxidase activity.

MATERIALS AND METHODS

Plant material

Lycopersicon esculentum Mill. (tomato), *Capsicum annuum* L. (pepper), *Phaseolus vulgaris* L. (bean) and *Vicia faba* L. (broad bean) seeds were obtained from May Agricultural Seed Company Inc. (Izmir/Turkey). Before the sowing process, 50 seeds for each plant were treated with distilled water for 5 h. Then, seeds were sowed in plastic pots (10 x 20 cm) containing a mixture of 1:2 perlite-peat. Seedlings were grown under greenhouse conditions (light/dark regime of 16/8 h at 25 ± 2°C, relative humidity 60 - 70%, photosynthetic flux density of (PAR) 350 mol m^{-2} s^{-1}) and organized with 5 replicates, each of which included 50 seedlings. Six weeks old plants were used for wastewater treatment process.

Chemicals

Pyrogallol, sodium phosphate, hydrogen peroxide, brilliant blue G-250, bovine serum albumin (BSA), phosphoric acid for enzyme and protein analyzes were obtained from Sigma Chemical Co. (St.Louis, MO, USA). Liquid nitrogen was obtained from HABAS (Bornova, Izmir/TURKEY) periodically.

Wastewater treatment

Wastewaters were taken from Dardanel Fisheries Company, Tekel alcoholic drink companies' wastewater treatment plants and from one station which is located in the middle of the Sarıçay River. All wastewaters analyses results were taken from Çanakkale Provincial Directorate of Environmental and Forestry. The water samples were collected in the sterilized one liter glass bottles; sampling period was once in a week for 3 times. The control plants were irrigated with distilled water during the experiment. For the wastewater treatment, Tekel, Dardanel and Sarıçay wastewaters were diluted with distilled water to 50 and 25%, respectively. Solutions were applied to the plants irrigation water in 100 mL. Wastewater treatment was applied twice in a week for three weeks. To analyze the elicitation response, leaves were taken in the 6 weeks old plants for total protein and peroxidase analysis.

Preparation of leaf extracts

Healthy terminal leaves were harvested in eight, ten true leaf stage and immediately placed in liquid nitrogen, freeze dried and lyophilized for 24 h at 4^0C and then were milled with a mortar and pestle to a fine powder. For the preparation of crude leaf extract, 0.2 g of crude extracts was homogenized with 2 ml of cold sodium phosphate buffer (0.05 M, pH 6.5), centrifuged at 20000 g for 15 min at 4^0C. After centrifugation, the supernatants were collected and their protein concentrations were determined according to Bradford (1976) using bovine serum albumin (BSA) as a standard (Bradford, 1976).

Protein and *enzyme analyses*

Plant specific proteins were analyzed according to Bradford (1976) with bovine serum albumin (BSA) as a standard (Bradford, 1976). Amount of total protein was measured spectrophotometrically at 595_{nm}. Peroxidase activity in the crude leaf extracts was assayed spectrophotometrically. 1 ml of assay mixture containing 0.05 M sodium acetate buffer (pH 6.5), 0.2 ml of 0.1 M pyrogallol, 0.1 ml of 90 mM H_2O_2 and an aliquot of the crude leaf extract containing 10 - 40 g proteins were mixed together immediately before detecting. The peroxidase enzyme activity was measured at 300 nm according to Kanner and Kinsella (1983). The kinetic enzyme reaction was allowed to proceed for 3 min and peroxidase measurements were taken in every 15 s with modified methods of Lurie et al. (1997). One unit of peroxidase activity is defined as $POD/300_{nm}$/ min/mg protein.

RESULTS AND DISCUSSION

Chemical composition of Dardanel Wastewater, Tekel Wastewater and Sarıçay River are given in Table 1. Total protein amount increased in *L. esculentum C. annuum, P. vulgaris* and *V. faba* according to our pot experiments. These plants were treated with wastewater series coming from Dardanel, Tekel and Sarıçay, compared to the control plants. Wastewater taken from Dardanel increased the total protein amount by 84.2% in bean, 72.7% in *V. faba*, while it slightly increased it by 45.4% in *L. esculentum* and 25% in *C. annuum*. Tekel wastewater increa-

Table 2. Total protein amounts in treated plants (± indicate for SE).

Plant	Control	Dardanel Wastewater		Tekel Wastewater		Sarıçay River	
	Protein (mg/ml)	Protein (mg/ml)	% impact	Protein (mg/ml)	% impact	Protein (mg/ml)	% impact
Lycopersicon esculentum Mill.	0.11 ± 0.01	0.16 ± 0.02	45.4	0.21 ± 0.01	90.9%	0.19 ± 0.02	72.7%
Capsicum annuum L.	0.04 ± 0.008	0.05 ± 0.005	25	0.08 ± 0.01	100%	0.11 ± 0.01	175%
Phaseolus vulgaris L.	0.19 ± 0.01	0.35 ± 0.02	84.2	0.39 ± 0.03	105.3%	0.34 ± 0.02	78.9%
Vicia faba L.	0.11 ± 0.01	0.19 ± 0.02	72.7	0.26 ± 0.01	136.3%	0.32 ± 0.02	190.9%

Table 3. Peroxidase activities in treated plants (± indicate for SE).

Plant	Control	Dardanel Wastewater		Tekel Wastewater		Sarıçay River	
	Peroxidase (min/mg/prot)	Peroxidase (min/mg/prot)	% impact	Peroxidase (min/mg/prot)	% impact	Peroxidase (min/mg/prot)	% impact
Lycopersicon esculentum Mill.	873 ± 27	525 ± 20	40%	171 ± 15	80%	537 ± 22	38.5%
Capsicum annuum L.	600 ± 21	480 ± 18	20%	500 ± 20	17%	491 ± 19	18.2%
Phaseolus vulgaris L.	505 ± 21	206 ± 10	59%	277 ± 12	45%	247 ± 11	51%
Vicia faba L.	927 ± 35	537 ± 22	42%	623 ± 26	33%	600 ± 24	35%

sed the total protein amount by 136.3% in *V. faba*, 105.3% in *P. vulgaris*, 100% in *C. annuum* and 90.9% in *L. esculentum*. Water taken from Sarıçay increased the total protein amount by 190.9% in *V. faba*, 175% in *C.annuum*, 78.9% in *P. vulgaris* and 72.7% in *L. esculentum*. Changes in total protein amounts were given in Table 2 for all treated plants.

Peroxidase enzyme activities decreased in all plant groups after wastewater treatment when compared with control plants. Dardanel wastewater treatment decreased the peroxidase activity by 59% in *P. vulgaris*, 42% in *V. faba*, 40% in *L. esculentum* and 20% in *C. annuum*. Tekel wastewater treatment decreased the peroxidase activity by 80% in *L. esculentum*, 45% in *P. vulgaris*, 33% in *V. faba* and 17% in *C. annuum*. Sarıçay River water treatment decreased the peroxidase activity by 51% in *P. vulgaris*, 35% in *V. faba*, 38.5% in *L. esculentum* and 18.2% in *C. annuum*. Changes in peroxidase activities were given in Table 3 for all treated plants.

The cellular stress response is a ubiquitous defense mechanism when cells are treated with different chemicals and wastewater. The induction of the stress response leads to expression of a group of proteins referred to as stress proteins, which are thought to protect the cell (Gabara et al., 2003). Activities of peroxidase expression have been shown in several plant systems to be altered by stress chemicals and infection (Herbette et al., 2003). Under stress conditions, the enhanced peroxidase activity in the intercellular spaces can probably lead to reduction of cell growth, stimulating cell wall stiffening. It has been observed that peroxidase induction is a general response of higher plants to the uptaking of toxic amounts of metals in roots and leaves of various species after application of toxic doses of Zn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+} (Sinha et al., 2008). Also different plants can give different responses against wastewater treatment in terms of total protein and peroxidase activities as in our research.

According to our research results, after the treatment with Dardanel, Tekel companies' wastewaters and Sarıçay River's water, the total protein amounts increased in all plant groups. In contrast, compared with the control plants peroxidase enzyme activity decreased in all plant groups after wastewater treatment. This shows us that the plant defense systems were blocked for the pioneer defense enzyme, peroxidase. According to the official water pollution control standards (Ministry of Environmental and Forestry Report, 2004), Sarıçay River has higher level of Biological oxygen demand (BOD), chemical oxygen demand (COD) and total phosphate values. These values effected the total protein increases in treated plants compared with control plants. Otherwise, Dardanel wastewater has also additionally higher levels of oil and grease. Tekel wastewater showed similarly peroxidase decreases along with Dardanel wastewater. However, Dardanel and Tekel wastewaters gave total protein increases in treated plants. Other researchers found that tannery sludge treated *Vigna radiata* L. varieties showed significantly increased protein contents with higher BOD and COD values (Sinha et al., 2008). It has been frequently reported that acid rain causes a decrease in GSH-Px and CAT activities starting 24 h after treatment while SOD and its izoenzyme CuZnSOD activities increased at 72 and 96 h (Gabara et al., 2003). Heavy metals present in strong wastewater were toxic and posed negative effects to both mangrove plants and soil

microbial activities (Yim and Tam, 1999).

In conclusion, there are some areas where freshwater resources increase or decrease according to rainfall changes due to climate change. A lot of hydrological and agronomic principles are available to improve the amount of water. Industrial wastewaters are used for agricultural irrigation and this is a good way to use this kind of water as a new water source. But in this case, the most important thing is to optimize the concentration of industrial wastewater, because one of the most common effects of wastewater application on the plants are growth inhibition or stimulation. In this way, the dangerous effects of wastewaters can be prevented, because some of the farmers are already using this kind of wastewater for irrigation in agricultural field all around the world.

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