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RESEARCH ARTICLE

Antioxidant biomarkers in *Gammarus pulex* to evaluate the efficiency of electrocoagulation process in landfill leachate treatment

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Abstract

The discharge of landfill leachate into the environment without effective treatment poses a serious threat for the aquatic ecosystems. This present study was undertaken to evaluate whether electrocoagulation process is efficient for treatment landfill leachate (LL) or not by using antioxidant biomarkers in *Gammarus pulex*. Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) activities and malondialdehyde (MDA) and glutathione (GSH) levels in *G. pulex* exposed to untreated, treated, and diluted rates 1/10 and 1/20 in both LL during 24 and 96 h were tested. Physiochemical characteristics of leachate (chemical oxygen demand, electrical conductivity, pH, phosphate, turbidity, NH_3, Cl^-, and color) were determined pre and post treatment. All physiochemical characteristics of LL decreased after treatment process. GSH-Px and CAT activities and GSH and MDA levels were increased in untreated groups when compared to control ($p < 0.05$). After treatment by electrocoagulation, MDA and GSH levels and CAT activities were returned to control values. In conclusion, the abilities of LL to stimulate oxidative stress in *G. pulex* have been proven. The results revealed that antioxidant parameters are useful biomarkers for determining the treatment efficiency of the electrocoagulation process.

Keywords *Gammarus pulex* · Antioxidant enzymes · GSH · MDA · Landfill leachate · Electrocoagulation process

Introduction

Landfill leachate is mostly generated due to biodegradation of the waste and the excess rainwater filtered through the solid waste layers. Migrating away from a landfill, small amounts of landfill leachate can cause critical pollution to the groundwater aquifer and adjacent surface waters (Li et al. 2006). Leachate includes many hazardous chemicals such as environmental persistence, toxicity, mobility, and lipophilicity, resulting in bioaccumulation in food webs (Fent 2004). Composition of leachates is usually characterized by a high level of organic matter. Leachates also contain some metal trace elements and different kinds of organic pollutants. Therefore, these cocktails of pollutants may have possible toxic and genotoxic effects (Olivero-Verbel et al. 2008).

There is crucial balance between reactive oxygen species (ROS) generation and their removal by antioxidant defense system in organisms. ROS are scavenged via antioxidant enzymes and non-enzymatic antioxidants (Hermes-Lima 2004; Halliwell and Gutteridge 2007). Lipid peroxidation is a chain reaction where oxidants create the breakdown of membrane phospholipids that have polyunsaturated fatty acids. Lipid peroxidation causes the damage to bio-membranes which can have important consequences for living organisms (Hermes-Lima 2004; Jemec et al. 2012). Pollutants could stimulate ROS production (Livingstone 2001). During oxidative challenge, cells mostly increase their levels of antioxidant enzymes such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) (Livingstone, 2001; Valavanidis et al. 2006). Lipid peroxidation and antioxidant defense mechanism have been successfully used as oxidative stress biomarkers in environmental studies and used in the assessment of effects of pollutants in aquatic environments (Livingstone 2001; Valavanidis et al. 2006).

Crustaceans are often used as bioindicators in many aquatic ecosystems (Rinderhagen et al., 2000). With their abundance

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in freshwater, their high ecological relevance, and their crucial role in the food chain, amphipods of the genus Gammarus are often employed in ecotoxicological studies (Kunz et al. 2010; Adam et al. 2010).

Electrochemical coagulation is an easy and effective method. It is the electrochemical production of destabilization agents that brings about charge neutralization for pollutant removal, and it has been utilized for wastewater treatment. Aluminum or iron plates are usually used as electrodes. Metallic ions that generated electrochemically from these electrodes can be hydrolyzed near the anode to produce a series of activated intermediates that can destabilize the finely dispersed particles present in the water or wastewater to be treated. The destabilized particles then aggregate to form flocks (Chen et al. 2002). Electrochemical coagulation (EC) is characterized for the removal of colloids, suspended solids, and other compounds (Oumar et al. 2016). One of the promising methods for treating wastewater is EC process. EC is applied to treat water containing dyes, oil wastes, food stuff wastes, organic matter from landfill leachates, chemical and mechanical polishing waste, synthetic detergent effluents, and mine wastes. Mechanism of electrocoagulation involves three steps: (a) creation of coagulants by electrolytic oxidation of the sacrificial electrode; (b) destabilization of the contaminants, particulate suspension, and refraction of emulsions, and (c) collection of the destabilized phases to form flocs (Saravanan et al. 2010).

In the present study, we aimed to investigate whether electrocoagulation process is efficient for treatment landfill leachate (LL) or not by using antioxidant biomarkers in *G. pulex*.

Materials and methods

Chemicals

Potassium chromate (K_2CrO_4), phenol (C_6H_6O), ammonium meta vanadate (NH_4VO_3), vanadate molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$, sulfuric acid (H_2SO_4), potassium dihydrogen phosphate (KH_2PO_4), sodium hydroxide (NaOH), and hydrochloric acid (HCI) were purchased from Sigma-Aldrich. Silver nitrate ($AgNO_3$), trisodium citrate ($Na_3C_6H_5O_7$), and sodium hypochlorite (NaClO) were purchased from Carlo Erba. Sodium nitroprusside ($Na_2[Fe(CN)_5NO]$) was purchased from Merck. Chemical oxygen demand (COD) kits were purchased from HACH Lange, COD digestion vials 0–1500 ppm.

Characteristics of treated and untreated leachate

Landfill leachate was taken from a young municipal landfill site from Bingol Province of Turkey. All chemical measurements for both untreated and treated leachates were determined according to standard methods (SM). Chloride (Cl^-) was analyzed with the use of SM 4500 Cl^-. Ammonia nitrogen (NH_3-N) was analyzed with the use of SM 4500-F. Phosphate (PO_4^{3-}) was analyzed with the use of SM 4500-P (Apha and WPCF, 2005). The COD was measured using a thermoreactor (HACH, DRB200) with the closed reflux method (5220D) (Apha and WPCF, 2005). Physicochemical parameters such as conductivity and pH were analyzed using a multiparameter (Thermo Orion 420A), while turbidity was measured using a digital portable turbidimeter (HACH 2100P). Color was measured according to Res methods using a UV/VIS spectrophotometer (Shimadzu 1800 UV/VIS spectrophotometer).

Animals and experimental procedure

Individuals of *G. pulex* were collected with handnets in the Munzur River from Tunceli, Turkey (39.156820 N, 39.499640 E). The organisms were rapidly transferred in plastic bottles to the laboratory where they were stocked in aerated 20-L aquaria in a climate-controlled room at 18 °C and a 12:12 light:dark cycle and fed willow leaves for 15 days before they were used for experiments. Organisms which are similar, adult, healthy, and active were selected for the study (De Lange et al. 2006). Each aquarium consists of 1 L water consisted of three replicates with 10 individuals. Experimental conditions for the acute test (96 h) used static tests. Organisms were not fed during the experiments. The organisms were checked per 24 h and dead individuals were counted and removed from the experiment aquarium. Inactivity was accepted as the criterion for death.

Five experimental groups were designed as control group (C); X1, untreated leachate (diluted 1/10 with tap water); X2, untreated leachate (diluted 1/20 with tap water); Y1, treated leachate (diluted 1/10 with tap water) by electrocoagulation; and Y2, treated leachate (diluted 1/20 with tap water) by electrocoagulation.

Experimental organisms in similar inter molt stage with about 10 mm in length were selected for the study (De Lange et al. 2006). The study was repeated three times. In each 1-L aquarium, 10 individuals were used. A total of 300 individuals were used for the five groups, in two periods (24 and 96 h) and three repetitions. Since there was not enough homogenate in 1 individual, so 10 individuals were mixed and a single sample was formed.

Biochemical analyses

The samples were weighed and homogenized by adding PBS buffer (salt solution buffered with phosphate) at a rate of 1/5 *w/v* and using a homogenizer with ice to measure antioxidant parameters. The samples were centrifuged at 17,000 rpm

for 15 min; the supernatants were kept in deep freeze at − 70 °C until their measurements were done. The concentration of GSH was performed by the method of Beutler et al. (1963) and expressed as nanomoles per gram tissue. The concentration of MDA was measured by the method of Placer et al. (1966) and expressed as nanomoles per gram tissue. SOD, CAT, and GSH-Px activities were conducted by using ELISA kit. The activities of SOD, CAT, and GSH-Px were determined by ELISA kits (catalog numbers, CAT 707002, SOD 706002, and GSH-Px 703102) purchased from The CAYMAN Chemical Company.

Electrocoagulation experiments

The design of the batch monopolar EC reactor was used in the present study. The electrolytic cell was made of plexiglass material and 1 L in volume, respectively. The electrode sets (anode and cathode) consisted of iron plates, with a dimension of 5 cm (width) × 6 cm (length) × 2 mm (depth), having a surface area of 30 cm^2. The batch electrochemical cell consisted of iron electrodes that were all individually connected to the dc power supply (AA Tech ADC-3303D, 0–3 A, 0–60 V). A working volume of 900 mL of leachate was used for all experiments ensured with a magnetic stirrer. The magnetic stirrer was placed above the cell and set at constant 200 rpm. The experiments were performed at room temperature (25 ± 2 °C) and without any electrolyte addition. Operational time was selected to be 120 min. The performance of the electrocoagulation treatments of leachate was improved for 17 mA/m^2 current density which was performed.

Statistical analyses

Data were analyzed using SPSS 18 software. One-way ANOVA and Duncan's multiple range tests were employed to evaluate the statistical differences in each application group (X1, X2, Y1, Y2) in the same hours ($^{abc}p < 0.05$). Two-tailed independent *T* test was used to compare the differences between exposure times (24 and 96 h) in the same application groups ($*p < 0.05$).

Results

In this study, physicochemical characteristics of LL were measured before and after electrocoagulation process and these parameters of LL were decreased after treatment (Table 1).

CAT, GSH-Px, and SOD activities and GSH and MDA levels of *G. pulex* exposed to all experimental groups (X1, X2, Y1, and Y2) during 24 and 96 h were illustrated in Fig. 1a–e.

MDA levels were higher in the untreated groups (X1, X2) ($p < 0.05$) than that in the control group for both 24- and 96-h exposure time. However, these levels in the treatment groups (Y1, Y2) were almost close to that in the control group ($p < 0.05$).

Table 1 Physicochemical characteristics of tap water and treated or untreated LL

Parameters	Groups				
	X1	X2	Y1	Y2	C
PO4–^{3} (mgl^{-1})	0.008	0.005	0.003	0.003	0.0025
NH3-N (mgl^{-1})	0.071	0.069	0.066	0.065	0.004
Turbidity (ntu)	76.2	48.3	1.66	1.10	0.51
pH	8.02	7.97	8.16	8.06	7.80
Conductivity (ms/cm)	4.63	3.34	5.67	3.94	0.36
Cl^- (mgl^{-1})	300	155	245	110	19
COD (mgl^{-1})	109	40	35	14	2
Color (m^{-1}) Abs_{436}	92.5	26.75	56	23.5	NC
Color (m^{-1}) Abs_{525}	69.0	22.5	42.25	20	NC
Color (m^{-1}) Abs_{620}	56.0	19.25	36.5	18.25	NC

NC no color was detected, *C* control group, *X1* untreated leachate (diluted 1/10 with tap water), *X2* untreated leachate (diluted 1/20 with tap water), *Y1* treated leachate by electrocoagulation (diluted 1/10 with tap water), *Y2* treated leachate by electrocoagulation (diluted 1/20 with tap water)

GSH levels for 24-h exposure time in all groups were lower than that in the control group ($p < 0.05$). These levels for 96 h in all groups were close to that in the control group ($p > 0.05$).

GSH-Px activities were decreased after treatment in the Y1 group for 24-h exposure time. The differences in GSH-Px activities of *G. pulex* between X1 and Y1 were found statistically significant for 24-h exposure time ($p < 0.05$) but these activities in all groups for 96-h exposure time were close to that in the control group ($p < 0.05$) except for the Y1 group.

CAT activities for 24-h exposure time in all groups increased compared to that in the control group except for Y2 ($p < 0.05$); however, for 96-h exposure time in all groups was close to that in the control group except for Y1 ($p < 0.05$).

SOD activities only decreased in the X1 group compared to that in the control group for 24-h exposure time ($p < 0.05$). Alterations in SOD activities in all groups were not statistically important for 96-h exposure time.

In the X1 group, there were no significant differences in CAT and SOD activities and GSH levels of *G. pulex* when exposure time was compared ($p > 0.05$); however, MDA levels and GSH-Px activities were observed to be statistically significant ($p < 0.05$). When exposure time was compared, no significant differences were found in all biomarkers in the X2 group ($p > 0.05$). In the Y1 group, there were no significant differences in GSH-Px activities and MDA levels of *G. pulex* when exposure time was compared ($p > 0.05$) while GSH levels and CAT and SOD activities were found to be statistically significant ($p < 0.05$). In the Y2 group, GSH-Px activities and GSH levels were statistically significant when exposure time was compared ($p < 0.05$).

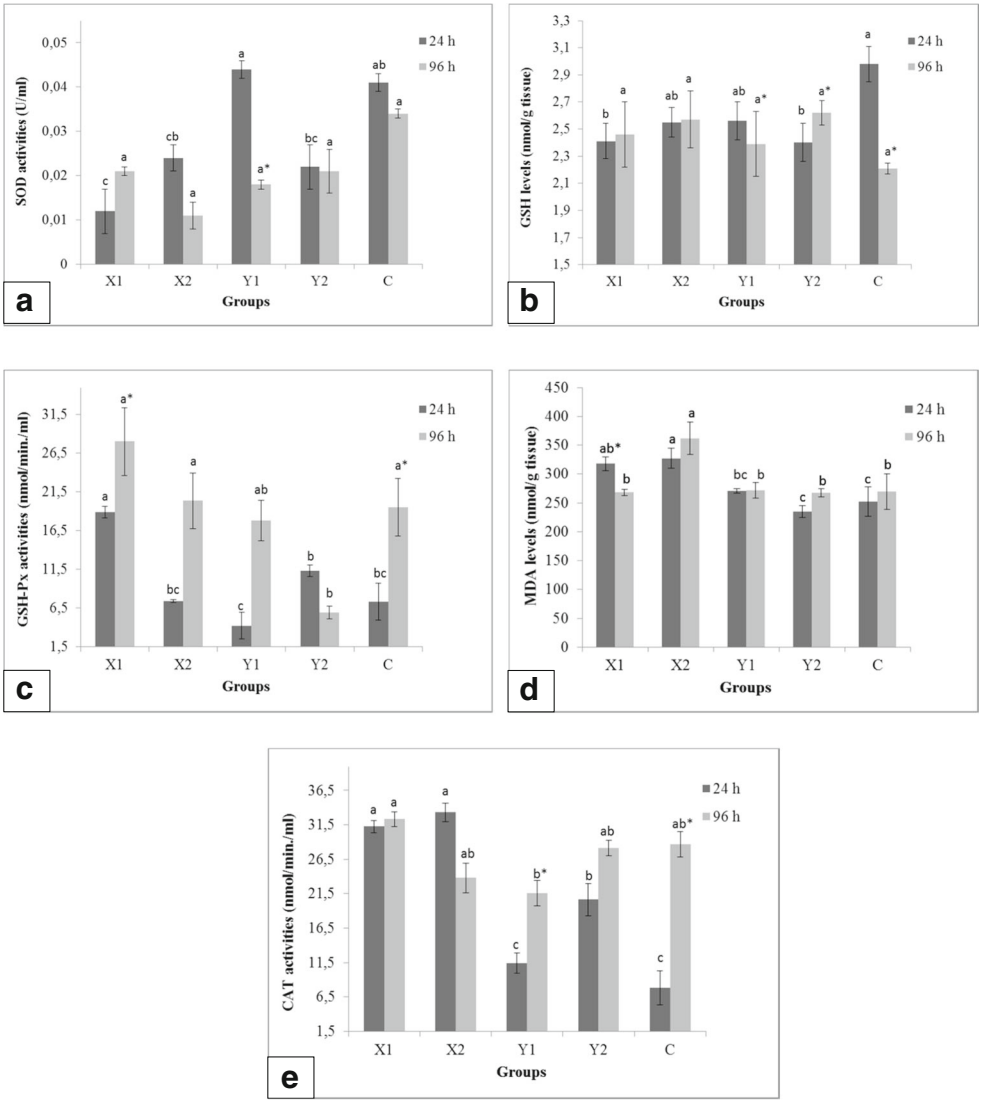

Fig. 1 **a** SOD (U/ml) activities, **b** GSH (nmol/g tissue) levels, **c** GSH-Px (nmol/min/ml) activities, **d** MDA (nmol/g tissue) levels, and **e** CAT (nmol/min/ml) activities of *G. pulex* exposed to different kinds of LL. Asterisk (*) shows statistical differences according to the two-tailed independent *T* test between different exposure time (24 and 96 h) in the same groups; $*p < 0.05$. Different letters on bar (a, b, c) show statistical differences of Duncan's multiple range test among all application groups in the same exposure time; abcp < 0.05. Values represent mean ± SE; $n = 10$

Discussion

Landfill leachate is a liquid that is produced by the rain which falls on the solid waste. The leachate contains high concentrations of ammonium, organic matter, toxic compounds, and heavy metals. COD, heavy metals, and other substances need to be reduced during leachate treatment (Kurniawan 2011). COD is known as the amount of oxidant consumed when samples were treated by the oxidant. It has been suggested as a general index of the level of organic pollution (Xi et al. 1996). In the present study, COD levels and other physiochemical parameters were decreased after electrocoagulation process (Table 1). In our study, high NH_3-N, conductivity, and chloride were found according to the EPA surface water quality criteria (EPA 2001). Studies have shown that $NH4^+$-N promotes the methane production when the concentration of $NH4^+$-N was lower than 0.4 g/L (Santos et al., 2004). NH_3 is toxic to many organisms. The importance of ammonia in toxicity is not a surprise. The 96-h LCs0 range from 0.32 to 3.10 mg of un-ionized NH_3-N/1 for several species of fish (Ruffler et al. 1981) and from 0.80 to 40 mg of un-ionized NH_3-N/I for 10 species of macro-invertebrates (Jean, 1991). The particular importance of ammonia in toxicity of landfill leachates to fish was reported by Cameron and Koch (1980). Deneuvy (1987) found for his part a more or less good correlation between ammonia and landfill leachate toxicity to daphnids, micro-algae, and bacteria of Microtox.

There is a relationship between the conductivity and chloride levels. Conductivity is an indicator of the abundance of the total concentration of the ions or the abundance of the dissolved inorganic species (Banar et al., 2006; Tatsi and Zouboulis, 2002). High conductivity value of the leachate may be attributed to high dissolved salts and metals in the leachate, metals which may be responsible for toxicity of leachates (Aiyesanmi and Imois, 2011).

In our study, the dark color of landfill leachate is the presence of high concentrations of humic substances which

represent the mostly organic compounds (Table 1) (Vedrenne et al., 2012; Qiu et al., 2016).

Recently, it has been demonstrated that leachates (complex chemical mixtures) are toxic and induced oxidative stress in aquatic organisms (Ali et al. 2004; Radetski et al. 2004). The toxicity level of leachate discharge to the aquatic environment can be determined by using aquatic organism as biological indicator (Raihana et al. 2014). Toxicological assessment of treated and untreated landfill leachate is essential to examine the effect of leachate discharged on the environment. Reduction in toxicity of the treated leachate helps in evaluating the effectiveness of the remediation strategy (Ganey and Boyd 2005). However, limited information is available that indicates oxidative stress inducing effect of LL. As far as what is known, there is no information in the literature on the effects of LL treated by electrocoagulation on SOD, CAT, and GSH-Px enzyme activities and GSH and MDA levels of *G. pulex.* In this study, a number of changes at the cellular level (SOD, CAT, and GSH-P_X activities and MDA and GSH levels) of *G. pulex* after untreated, treated, and diluted rates 1/10 and 1/20 in both LL during 24 and 96 h were tested to reveal the electrocoagulation process efficiency in model organism (*G. pulex*).

Pollution in aquatic environments can enhance ROS production resulting from imbalance between ROS concentrations and antioxidant defense system, leading to toxic effects such as lipid peroxidation (Regoli et al. 2004). Key antioxidant enzymes and non-enzymatic antioxidants have been shown to be influenced by various single pollutants known to increase ROS levels (Valko et al. 2006; Ryter et al. 2007). The activities of oxidative stress, detoxification, and neurotoxicity biomarkers have been used in Gammarus (Demirci et al. 2017). We used antioxidant parameters in our study as a biomarker. Increment of glutathione and glutathione-dependent enzymes is defined as the primary response to exposure to toxic compounds with potential of inducing oxidative stress (Lindesjoo et al. 2002; Oruc and Uner 2002). The change in GSH-Px activity is often accompanied by changes in the level of GSH, which is the co-substrate for H_2O_2 decomposition by GSH-Px (Sies 1999). Kutlu and Susuz (2004) observed slightly inhibited activity of GSH-Px after exposure to lead acetate in invertebrate, *G. pulex*. In the present study, similar GSH-Px and GSH induction were determined in *G. pulex* exposed to different kinds of untreated leachate (Fig.1b, c).

Increased SOD, GSH-Px, and CAT activities were found in hearts, kidneys, and spleens of mice in response to leachate (Li et al. 2006). It was demonstrated that the pesticide mixture induces synergistic interactions leading to more oxidative stresses and thus induces CAT and SOD enzymes in *Gammarus kischineffensis* (Demirci et al., 2017). It was found that elevated antioxidant stress enzyme activities, e.g., superoxide dismutase (SOD) and catalase (CAT), were detected in Vicia root tissues even at the lowest tested leachate concentration (Radetski et al. 2004). Sub-lethal Cd exposure concentrations and durations significantly affect the levels of antioxidative defense enzymes (SOD, CAT, and GSH-Px), MDA, and protein content in *G. pulex*. Cd accumulation and MDA content increased depending on the exposure concentration and duration (Duman and Kar. 2015). Similarly in our study, CAT activities and MDA levels were also increased in untreated groups when compared to control ($p < 0.05$) (Fig. 1d, e). CAT was stimulated to scavenge the H_2O_2 due to the increased pollutant concentration at higher concentration of leachate. The increased antioxidant enzymes and MDA levels show that biological membranes were attacked by free radicals and the antioxidant enzymes were induced to prevent against hyperoxia and lipid peroxidation, which was possible metabolic adaptation to the exposure to LL and was defense against oxidative damage (Li et al. 2006).

Superoxide dismutase is the first enzyme of antioxidant defense system (Vijayavel et al. 2004) by accelerating the dismutation of superoxide (O_2) to H_2O_2. It damages the membrane and biological structures. Decreased SOD activity was found in untreated groups compared to control; the decrease of SOD indicates that this antioxidant enzyme was inhibited; its protective effects against free radicals were reduced. After treatment by electrocoagulation, MDA and GSH levels were returned to control values (Fig.1b, d).

The landfill leachate toxicity to the Asian clam *Corbicula fluminea* was investigated by using biomarkers and variability in biomarker responses among leachate concentrations was observed (Oliveira et al. 2014). The toxicological effects of municipal landfill leachate on *Vicia faba* were studied in different seasons and toxicity study was performed via various antioxidant parameters. A dose-dependent elevation in the MDA level and inhibited antioxidant enzyme activities were observed (Gupta and Rajamani 2015). In the present study, we also found different biomarker responses depending on leachate concentrations and exposure times. Exposure time is important in determining cellular responses because the intracellular redox state is presumed to be altered according to the duration of the exposure (Ishihara and Shimamoto, 2007). Exposure concentration and duration have strong synergistic effects on antioxidant enzyme activity (Duman and Kar, 2015).

Conclusion

In conclusion, it has been demonstrated that LL stimulates oxidative stress and the positive correlations between antioxidant responses. Different LL concentration affected antioxidant biomarkers in different levels in *G. pulex.* Also, the biomarkers such as SOD, CAT, GSH-Px, MDA, and GSH clearly revealed metabolic changes after LL exposure but only MDA and GSH levels of *G. pulex* confirmed the LL treatment efficiency of electrocoagulation process.

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