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THE EFFECT OF BATTERY RECYCLING INDUSTRY SOLID WASTE LEACHATE ON ANTIOXIDANT STATUS OF *TRIGONELLA FOENUM-GRAECUM*

Jeba Sweetly, D*[1]., K. Sangeetha[2] and B. Suganthi[2]
[1]Department of Biochemistry, [2]Department of Biotechnology,
Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.
*E-mail: sweetly5647@gmail.com

ABSTRACT

Effect of battery recycling industry solid waste leachate on the growth and antioxidant *status of Trigonella foenum-graecum* was investigated. Plants have played a crucial role in maintaining human health and improving the quality of human life for thousands of years. Plants were grown in four dilutions of leachate and the results were compared with the control plants grown simultaneously in the same condition. Enzymic and Non-enzymic antioxidants were analyzed in the coriander leaves. The concentration of Superoxide dismutase, Glutathione peroxidase, Ascorbic acid, Reduced glutathione were studied in the leachate and control growing plants. This study proves that coriander should not be grown in the soil having highest level of battery recycling industry solid waste for maintaining human health and the environment.

Keywords: Antioxidant, battery recycling, solid waste leachate, coriander

1. INTRODUCTION

Heavy metals induce oxidative stress by generating free radicals and toxic reactive oxygen species. These species react with lipids, proteins, pigments and nucleic acids and cause lipid peroxidation, membrane damage and inactivation of enzymes, thus affecting the cell viability. The deleterious effects resulting from the cellular oxidative state may be alleviated by the enzymatic and non enzymatic antioxidant machinery of the plant (Sharma and Agrawal, 2005).

One of the major mechanisms behind heavy metal toxicity has been attributed to oxidative stress. Toxic metals increase production of free radicals and decrease availability of antioxidant reserves to respond to the resultant damage. A growing amount of data provide evidence that metals are capable of interacting with nuclear proteins and DNA causing oxidative deterioration of biological macromolecules (Leonard, 2004).

The battery industry represents one of the most important and growing sectors where the use of non-toxic and non-hazardous substitute material has not rapidly developed. As regulations increase and concern for the environment and human health becomes more prevalent, the fate of toxic and hazardous materials in the environment should be more carefully considered. Exide's network spreads throughout India and its factories are geographically distributed at strategic locations around the country. Exide industry is one of the leading companies towards manufacturing of lead-acid batteries nationally and internationally (Rahangdale *et al*., 2012).

The maximum allowable concentration of lead is 0.05 mg L-1 for drinking water and 0.2 mg L-1 for effluent discharge. Lead is used for manufacturing of storage batteries, due to its characteristic properties: conductivity, corrosion resistance and reversibility of the reaction between lead, lead oxide and sulfuric acid (Dermentzis *et al*., 2012).

Plants are rich sources of natural antioxidants that play a vital role in the prevention or progression of the degenerative diseases. The consumption of fruits, vegetables and herbs rich in antioxidants is associated with a decline in the incidence of degenerative diseases and cancer (Harish *et al*., 2005).

Plants possess two very efficient antioxidant defense systems: the enzymic which includes catalase, peroxidase, superoxide dismutase, polyphenol oxidase, glutathione reductase and the non enzymic antioxidant defense systems such as ascorbic acid, glutathione, tocopherol and carotenoids. Both allow scavenging of reactive oxygen species leading to protection of plant cells from oxidative damage (Blokhina *et al*., 2003; Gratao *et al*., 2005). Indeed, activities of antioxidant enzymes have been detected in various cellular organelles of various plant species. These antioxidant enzymes were found in various compartments of the plant leaf cell, e.g: superoxide dismutase (SOD) found in chloroplasts (Mittova *et al*., 2000). It is found that people who eat fruits and vegetables rich in polyphenols and anthocyanins have a lower risk of cancer, heart disease and some neurological diseases (Stanner *et al*., 2004). Antioxidants can cancel out the cell damaging effects of free radicals. These compounds might prevent conditions such as macular degeneration, suppressed immunity to poor nutrition and neurodegeneration which is caused by oxidative stress (Wang *et al*., 2005).

The term antioxidant originally was used to refer to a chemical that prevented the consumption of oxygen. Research into how vitamin E prevents the process of lipid peroxidation led to the identification of antioxidants as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells (Wolf, 2005). Heavy metals induce oxidative stress by generating free radicals and toxic reactive oxygen species. These species react with lipids, proteins, pigments and nucleic acids and cause lipid peroxidation, membrane damage and inactivation of enzymes, thus affecting the cell viability. The deleterious effects resulting from the cellular oxidative state may be alleviated by the enzymatic and non enzymatic antioxidant machinery of the plant (Mittler 2002; Sharma and Agrawal, 2005).

Fenugreek is one of the oldest medicinal plants, originating in India and Northern Africa. An annual plant, fenugreek grows to an average height of two feet. This plant use for blood lipids and sugar decreasing in diabetic and non diabetic people and has antioxidant and antibacterial activity. This plant decreases body fats and effective on obesity. This plant are used in therapy atherosclerosis (Nandini *et al*., 2007), rheumatism (Amit *et al*., 2010), sugar lowering (Gupta *et al*., 2001), blood lipids lowering (Xue *et al*., 2007), appetizer and contain antioxidant activity (Bukhari *et al*., 2008).

A major defence mechanism involves the antioxidant enzymes, which includes SOD, catalase and glutathione peroxidase (GPx), which converts active oxygen molecules into non toxic compounds (Merlin and Parthasarathy, 2011).

Hence, in the present study antioxidant status of *Trigonella foenum-graecum* plant was investigated under different concentration of battery recycling industry solid waste leachate.

2. MATERIALS AND METHODS

2.1. Collection of solid waste

The Battery recycling industry solid waste was collected from Industrial area near kuruchi, Coimbatore, Tami Nadu.

2.2. Preparation of leachate from battery recycling industry solid waste

Leachates were prepared by adding 100 g of solid waste to 1L of demineralized water (10% w/v), followed by continuous shaking for 24 h at ambient room temperature (25±1 10°C) following the were removed by centrifugation at 3000 rpm for 15 min and the leachate samples were preserved in screw-capped bottles at 4± 10°C (Coya *et al*., 2000) for further use.

2.3. Randomized block design

Pot culture experiment was conducted for a period of 60 days. Red soil and sand free from pebbles and stones were mixed in the ratio of 3:1. 10 kg of the mixture was filled it the individual pots. The seeds of coriander selected for the study were collected form Department of pulses, Tamil Nadu Agricultural University, Coimbatore. The study carried out at Avinashilingam University was laid out in a complete randomized block design consisting of 4 treatments with a control. Each experiment was replicated thrice. The seeds were sown in different pots in five different dilutions as follows and used for the pot culture study.

Control - plants grown in normal water
25% - 25ml leachate + 75 ml water
50% - 50ml leachate + 50 ml water
75% - 75ml leachate + 25 ml water
100% - 100ml leachate

There was no germination observed when seeds were grown with 100% leachate. Hence the present study continued with 25%, 50% and 75% leachate. The biochemical parameters in the leaves of selected plant which were subjected to the various treatments as indicated in table 1 were analyzed for a period of 20th, 40th and 60th days.

Table 1. Biochemical parameters analysed

Characteristics	Method of analysis	References
Superoxide dismutase	Spectrophotometry	Misra and Fridovich, 1972
Glutathione peroxidase	Spectrophotometry	Rotruck *et al*., 1973
Ascorbic acid	Spectrophotometry	Roe and Kuether, 1953
Reduced glutathione	Spectrophotometry	Moron *et al*., 1979

2.4. Statistical analysis

The results for biometric and biochemical parameters were expressed as mean±S.D. The results were subjected to TWO WAY ANOVA using Sigma Stat Version 3.0. A value of $p < 0.05$ considered to be highly significant.

3. RESULTS AND DISCUSSION

Antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals or oppose their actions. There are two main categories of antioxidants (enzymic and non enzymic) whose role is to prevent the generation of free radicals that are generated (Priya and Surapaneni 2008; Kaur *et al.*, 2008).

Heavy metals induce oxidative stress by generating free radicals and toxic reactive oxygen species. These species react with lipids, proteins, pigments and nucleic acids and cause lipid peroxidation, membrane damage and inactivation of enzymes, thus affecting the cell viability. The deleterious effects resulting from the cellular oxidative state may be alleviated by the enzymatic and non enzymatic antioxidant machinery of the plant (Sharma and Agrawal, 2005).

A major defence mechanism involves the antioxidant enzymes, which includes SOD, catalase and glutathione peroxidase (GPx), which converts active oxygen molecules into non toxic compounds (Merlin and Parthasarathy, 2011). In the present study, selected enzymic and non enzymic antioxidants were analysed in the plants grown with untreated leachate.

Table 2. Superoxide dismutase activities in the leaves of *Trigonella foenum-graecum.*

	Superoxide dismutase (Units/g)		
	20th day	40th day	60th day
Control	0.062±0.002	0.068±0.004	0.052±0.001
25% Leachate	0.059±0.002	0.067±0.001	0.043±0.001
50% Leachate	0.054±0.001	0.055±0.001	0.039±0.001
75% Leachate	0.045±0.001	0.050±0.001	0.036±0.001
CD (*P<0.05*)		0.0018	

From t he table 2 and figure 1 shows the superoxide dismutase activity in the leaves of Fenugreek grown in red soil with and without the various leachate samples. Among the various treatments, control recorded higher enzymic activity followed by 25% leachate, while 50 and 75% leachate exhibited lower activity of the enzyme.

He and Goa, 2008 have also reported a decline in the activity of SOD, CAT and POD after flowering stage in the plant *Chimonathus praecox*, which is similar to our observation.

Table 3. Glutathione peroxidase activities in the leaves of *Trigonella foenum-graecum*

	Glutathione peroxidase (Units/g)		
	20th day	40th day	60th day
Control	0.157±0.001	0.163±0.006	0.142±0.002
25% Leachate	0.139±0.001	0.152±0.002	0.132±0.006
50% Leachate	0.133±0.008	0.146±0.003	0.130±0.002
75% Leachate	0.120±0.002	0.127±0.001	0.112±0.002
CD(*P*<0.05)		0.0018	

From table 3 and figure 2 it is shown that glutathione peroxidase activity was maximum in fenugreek grown with 25% leachate. There was a decrease in plant grown with 50% and 75% leachate. However the enzymic antioxidant levels were found to be reduced on 60th day in plants grown with various leachate concentrations.

Ascorbic acid has antioxidant activity and it reduces oxidizing substances such as hydrogen peroxide (Duarte and Lunec, 2005). It can also reduce metal ions which lead to the generation of free radicals through the Fenton reaction (Valko *et al.*, 2005).

Table 4. Ascorbic acid content in the leaves of *Trigonella foenum-graecum*

	Ascorbic acid (mg/g)		
	20th day	40th day	60th day
Control	0.036±0.002	0.038±0.001	0.033±0.001
25% Leachate	0.026±0.002	0.033±0.001	0.024±0.001
50% Leachate	0.022±0.001	0.028±0.002	0.021±0.001
75% Leachate	0.020±0.001	0.024±0.002	0.019±0.001
CD(*P*<0.05)		0.002	

From Table 4 and figure 3, the ascorbic acid content was maximum in the control group followed by 25% leachate, whereas a significant reduction was observed in 50% and 75% leachate.

Table 5. Reduced glutathione content in the leaves of *Trigonella foenum-graecum*

	Reduced glutathione (nmoles)		
	20th day	40th day	60th day
Control	0.151±0.001	0.164±0.002	0.139±0.001
25% Leachate	0.146±0.001	0.162±0.002	0.123±0.004
50% Leachate	0.139±0.002	0.151±0.002	0.107±0.003
75% Leachate	0.105±0.006	0.133±0.001	0.091±0.003
CD(*P*<0.05)		0.198	

From table 5 and figure 4 it is clear that fenugreek plant grown with normal water showed elevated levels of reduced glutathione in both the plants with treated and 25% leachate. 50% and 75% leachate showed minimum amount of glutathione reductase when compared to normal control showing a negative correlation between the non enzymic antioxidant content and leachate concentration.

4. CONCLUSION

Based on these results the farmers around the locality should be properly educated about the beneficial effect of battery recycling industry leachate in agriculture and it would facilitate reduction in pollution load on environment. It is therefore conceivable that illegal dumpsite should be closed and landfills with effective leachate collection system should be built and they should be located in the outskirts of city far away from dwelling houses. A pot culture experiment was carried out to study the effect of the untreated and control samples of leachate on the growth attribute of the selected plant fenugreek. Various biochemical parameters such as enzymic and non enzymic antioxidants were determined. The plants grown with 25% leachate samples showed better growth characters which were comparable with that of control. Since the application of 25% leachate samples resulted in better growth of the selected green leafy vegetable coriander. Hence these leachates on a large scale can be used for irrigation purposes.

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