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# Study the stress of treated pharmaceutical effluent on Peroxidase, MDA and Proline content of Brown gram (*Cicer arietinum*) and Moong (*Vigna radiata*)

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## Abstract

*The influence of treated pharmaceutical effluent was studied on Peroxidase activity, MDA and Proline content of Cicer arietinum and Vigna radiata seedlings. Seedlings were raised in petri plates soaked with different dilution of treated pharmaceutical effluent (100%, 75%, 50%, 25% and 0%). The oxidative stress markers Peroxidase activity, MDA and Proline content were analyzed on 7th day old seedlings. The Peroxidase activity, MDA and Proline content was least in control (100%) as compared to other dilution showing that the treated pharmaceutical effluent had caused oxidative stress in seedlings of Cicer arietinum and Vigna radiata.*

**Keywords:** Pharmaceutical effluent, *Cicer arietinum*, *Vigna radiata*, seedlings, Peroxidase activity, MDA, Proline and oxidative stress.

## Introduction

Pollution due to discharge of industrial waste has become a serious problem in most of the areas of our country. Usage of effluents not only prevents the environmental pollution, but also serves as an additional potential source of liquid fertileness to crops, as it contains organic, inorganic compound and trace metals. That is why ecologists have started to think of various remedial measures that the waste of yesterday could become a useful product of today with reference to pollution[1]. Rapid industrialization and urbanization is one of the reasons of environment pollution. It is a peak time now to make our environment healthy to live.

Water resources are most often polluted by industrial effluents[2]. Water is polluted due the discharge of large quantities of industrial wastes affecting the aquatic life. Polluted water may be defined as addition of substance which affects the quality of water. India is an agricultural country where the growth and development is primarily depends on agriculture so here the utilization of industrial effluent for irrigation could be done to keep the environment clean and healthy. Besides the negative aspects of industrial effluent it's our duty to make them beneficial for agriculture and society.

## Material and Methods

**Collection of Sample:** The effluent of pharmaceutical industry producing folic acid was used for the present study and the analysis of pharmaceutical effluent was done at Pollution control board of Indore shown in table-3.

**Seed Materials:** For present study healthy seeds of *Cicer arietinum* and *Vigna radiata* having uniform shape and size were used.

**Germination Study:** The five different dilution of treated pharmaceutical effluent viz 100%, 75%, 50%, 25% and 0% were used in the study. Seeds of *Cicer arietinum* and *Vigna radiata* were sterilised using 0.1% $HgCl_2$ solution for 5 min and then were washed with distil water. Seeds were allowed to germinate for 24 hrs. Germinated seeds were then transferred in petriplates lined with Whatmann filter paper no.1. 3ml of respective dilution was added to moisten filter paper in each petriplates and every day at the first day of experiment and then 2 ml of respective dilution was added for consecutive 6 days. Three sets in each dilution were maintained along with the control for comparison. On the 7th day, peroxidase activity, MDA and proline content were determined.

**Peroxidase Activity:** The method given by J.B.Summer *et al.*, [3] was used to measure Peroxidase activity.

**Calculation:** The enzyme's specific activity is expressed as units/min/mg protein. An increase in OD by 1.0 under standard conditions is considered as one unit of enzyme.

**Malondialdehyde Estimation:** The MDA was calculated using the method of R.L. Heath and L.Packer[4].

**Calculation:** MDA concentration was calculated using the extinction coefficient of 155 $mM^{-1}cm^{-1}$ for MDA at 532nm using the formula-

$$A = \varepsilon l c$$

Where, A= Absorbance at specific wavelength. Ɛ= Extinction coefficient. l= length of cell (1 cm) c= concentration.

**Proline Estimation:** Proline was estimated according to L.S.Bates *et al.*,[5].

**Calculation:** The proline content is expressed as: µmole per g tissue= µ g proline/ml x ml toluene/115.5 x 5 g of sample, where the molecular weight of proline is 115.5.

## Results and Discussion

Table 1 is showing effect of treated pharmaceutical effluent on Peroxidase activity, MDA and Proline content of *Cicer arietinum*.

The peroxidase activity in untreated seedlings of *Cicer arietinum* was 78.16±.76 while the peroxidase activity of seedlings treated with 75%, 50%, 25% and 0 % dilution were 78.17±.84, 79.50±.86, 79 .50±0.86 and 79.83±.076 respectively. The maximum increase was (2.09%) found at 0% dilution.

MDA content in untreated seedlings of *Cicer arietinum* was .0051±.0014 while the MDA content of seedlings treated with 75%, 50%, 25% and 0 % dilution were 0.0055±.0.0004, 0.0058±0.0002, 0.0059±0.0002 and 0.0059±0.0003 respectively. The maximum increase were (13.55%) found at 25% and 0% dilution.

Proline content in untreated seedlings of *Cicer arietinum* was 196.66±7.63 while the proline content of seedlings treated with 75%, 50%, 25% and 0 % dilution were 220.66±2.31, 263.33±7.52, 276.66.±3.22 and 280.00±2.04 respectively. The maximum increase was (29.76%) found at 0% dilution.

Table 2 is showing effect of treated pharmaceutical effluent on on Peroxidase activity, MDA and Proline content of *Vigna radiata*.

The peroxidase activity content in untreated seedlings of *Vigna radiata* was 40 .06±3.46 while the peroxidase activity of seedlings treated with 75%, 50%, 25% and 0 % dilution were 52.5±1.80, 57.16±0.57, 60.83±1.04 and 77.33±1.75 respectively. The maximum increase was (48.19%) found at 0% dilution.

MDA content in untreated seedlings of *Vigna radiata* was 0.0006±0.0001 while the MDA content of seedlings treated with 75%, 50%, 25% and 0 % dilution were 0.0007±0.0001, 0.0007±0.0001, 0.0007±0.0001 and .0008±0.0001 respectively. The maximum increase was (25.00%) found at 0% dilution.

Proline content in untreated seedlings of *Vigna radiata* was 141.66±2.88 while the proline content of seedlings treated with 75%, 50%, 25% and 0 % dilution were 271.66±2.88, 286.66±2.88, 310.66±1.15 and 321.61±2.88 respectively. The maximum increase was (56.07%) found at 0% dilution.

In the present study there was a significant increase in peroxidase activity of both *Cicer aritenum* and *Vigna radiata* compared to 100% dilution. In legume leaves and seeds antioxidants level was studied in detail[6]. The present study result was supported by the some workers who reported that peroxidase activity of the leaves and seeds of *Vigna radiata* and *Vigna mungo* showed significant increase with increase in effluents concentration[7]. Similarly some researchers observed an increase in the activity of peroxidase in root, stem and leaf of *Beta vulgaris* plants grown in sewage sludge amended pots[8]. Some scientists found contradict results of decreased peroxidase enzyme activities after wastewater treatment in *Lycopersicon esculentum* M., *Capsicum annuum* L., *Phaseolus vulgaris* L. and *Vicia faba* L. when compared with control plants[9].

The oxidative lipid injury is estimated using MDA as marker whose concentration varies in response to biotic and abiotic stress[10]. MDA is generally accepted markers of oxidative stress which is the product of lipid peroxidation[11]. The level of MDA in *Cicer arietinum* and *Vigna radiata* was insignificantly increased with decrease in dilution %. The result was in agreement with the result of some workers who showed that the plants grown at wastewater irrigated sites showed higher MDA concentration, an indicator of lipid peroxidation as compared to those grown at ground water irrigated site[12]. These data are in concord with the results from study on *Bruguiera gymnorrhiza*[13].

**Table-1**
**Showing effect of treated pharmaceutical effluent on Peroxidase activity, MDA and Proline content of *Cicer arietinum***

| S. No. | Dilution | Parameter | | |
|---|---|---|---|---|
| | | Peroxidase Activity ( Units/min/g ) | MDA Content (Mm/mg) | Proline Content (µmole per g) |
| 1 | 100% | 78.16±0.76 | 0.0051±0.0014 | 196.66±7.63 |
| 2 | 75% | 78.17±0.84* | 0.0055±0.0004$^{ns}$ | 220.66±2.31$^{ns}$ |
| 3 | 50% | 79.50±0.86* | 0.0058±0.0011$^{ns}$ | 263.33±7.52$^{ns}$ |
| 4 | 25% | 79.50±0.86* | 0.0059±0.0002 $^{ns}$ | 276.66±3.22$^{ns}$ |
| 5 | 0% | 79.83±0.76* | 0.0059±0.0003 $^{ns}$ | 280.00±2.04* |

* Significant ($p<0.05$), **very significant ($p<0.01$) and ns not significant ($p>0.05$) Changes when compared with 100% dilution.

**Table-2**
**Showing effect of treated pharmaceutical effluent on on Peroxidase activity, MDA and Proline content of *Vigna radiata***

| S. No. | Dilution | Parameter | | |
|---|---|---|---|---|
| | | Peroxidase Activity (Units/min/g ) | MDA Content (Mm/mg) | Proline Content (µmole per g) |
| 1 | 100% | 40.06±3.46 | 0.0006±0.0001 | 141.66±2.88 |
| 2 | 75% | 52.5±1.80** | 0.0007±0.0001 ns | 271.66±2.88** |
| 3 | 50% | 57.16±0.57** | 0.0007±0.0001 ns | 286.66±2.88** |
| 4 | 25% | 60.83±1.04** | 0.0007±0.0001 ns | 310.66±1.154** |
| 5 | 0% | 77.33±1.75** | 0.0008±0.0001 ns | 321.66±2.88** |

*significant ($p<0.05$), ** very significant ($p<0.01$) and ns not significant ($p>0.05$) Changes when compared with 100% dilution.

**Table-3**
**Result of analysis of pharmaceutical effluent used in present study**

| Parameters | Methods | Treated Pharmaceutical Effluent | BIS Limits is 2490-2009 |
|---|---|---|---|
| Colour | - | Transparent | - |
| Odour | - | Offensive | - |
| Ph | Electrometric | 7.52 | 5.5-9.5 |
| Acidity | Titrimetric | ND | - |
| Alkalinity | Titrimetric | 330mg/l | - |
| Turbidity | Turbidimetric | 112.3ntv | - |
| Total dissolve solid | Gravimetric | 1250mg/l | 2100mg/l |
| Total solids | Gravimetric | 1306mg/l | 10mg/l |
| Electrical conductivity | Potentiometric | 2084µmho | Not mentioned |
| COD | Reflux | 217.36mg/l | 250mg/l |
| BOD | - | 46mg/l | 30mg/l |
| Fe | - | .139mg/l | - |
| Sulphate | Turbidimetric | 41.70mg/l | 1000mg/l |
| Chloride | Argenometric | 460mg/l | - |
| Total nitrogen | Kjeldahl | 50.42mg/l | - |

In the present study the proline content of both *Cicer arietinum* and *Vigna radiata* was significantly high in all dilution as compared to 100% dilution. The maximum increase was seen at 0% dilution. The proline content was higher in plants grown at wastewater irrigated sites than ground water irrigated site[12].

## Conclusion

The present study revealed negative impact of treated pharmaceutical effluents on seedlings. Increase in MDA level, peroxidase activity and proline content showed the oxidative stress was caused by pharmaceutical effluent. The maximum increase was found at 0% dilution i.e with undiluted effluents. Hence treated effluents of pharmaceutical industry can be used for irrigation only after proper dilution to solve the problem of scarcity of water.

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