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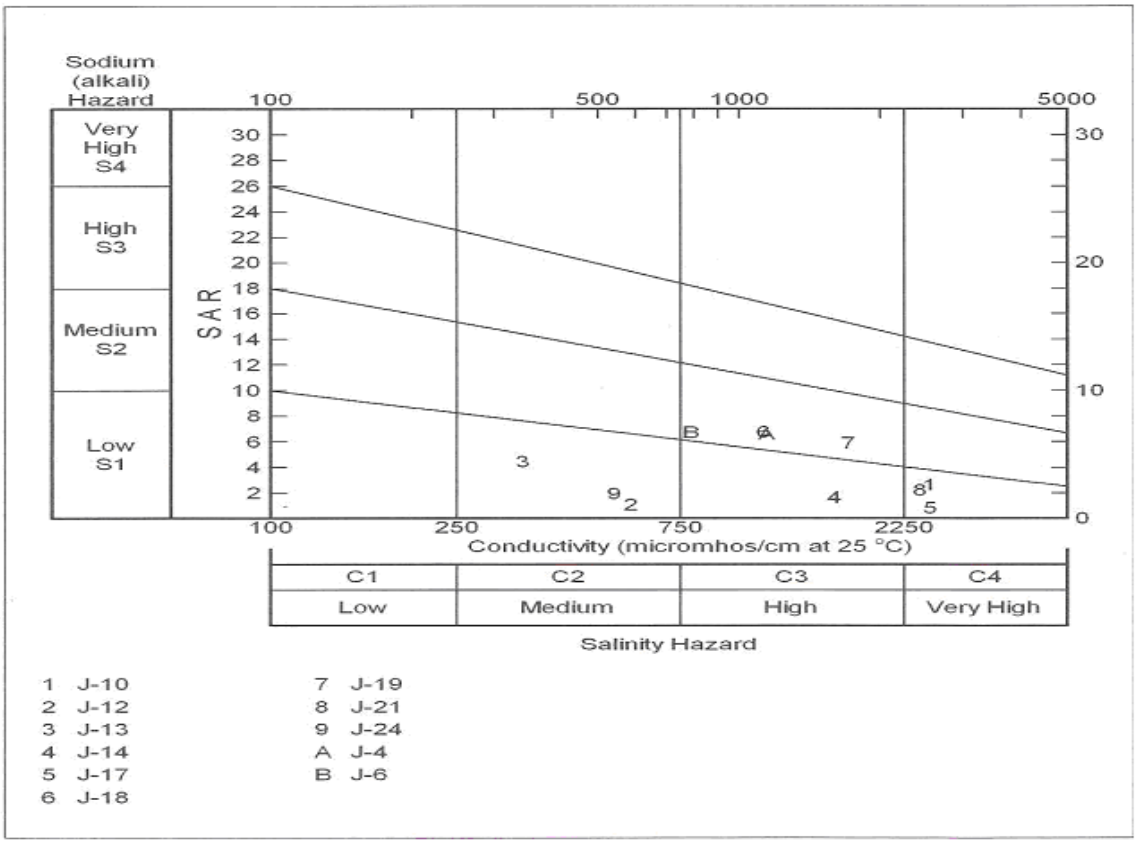


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# INFLUENCE OF TEXTILE MILL WASTEWATER IRRIGATION ON THE GROWTH OF SORGHUM CULTIVARS

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**Abstract.** The aim of the presented study was to evaluate the suitability of textile mill wastewater (treated and untreated) at different concentrations (0, 6.25, 12.5, 25, 50, 75, and 100%) for irrigation purposes. Effect of textile mill wastewater on germination, delay index, physiological growth parameters and plant pigments of two cultivars of sorghum was studied. The textile effluent did not show any inhibitory effect on seed germination at lower concentration (6.25%). The other reported plant parameters also followed the similar trend. Seeds germinated in 100% effluents but did not survive for longer period. It has also been concluded that effect of the textile effluent is cultivar specific and due care should be taken before using the textile mill wastewater for irrigation purpose.
**Keywords**: *Textile mill wastewater, sorghum cultivars, germination (%), delay index, plant biomass.*

## Introduction

India has a large network of textile industries of varying capacity. Textile industries have been placed in the category of most polluting industries by the Ministry of Environment and Forests, Government of India. Textile industries in India were initially centred around big cities like Ahmedabad, Mumbai, Chennai, Coimbatore, Bangalore and Kanpur. Now the industries are well developed and a large number of small textile processing units are scattered all over the country. Wet processes like bleaching, dyeing and screen printing are being carried out by these industries. Textile processes requires large volumes of fresh water after the cloth processing operations. The wastewater volume varies from mill to mill. The combined wastewater volume from Indian textile mills lies in the range of 86 to 247 litres with an average 172 litre per kg of cloth produced. Their effluents constitute a major part of the total industrial effluents in India. On an industrial scale the effluents resulting from the dyeing and printing operations of textile mills are managed through primary or secondary wastewater treatment.

A huge amount of effluent from textile mills is being discharged on land or into water courses. This effluent is characterized by high biological oxygen demand (BOD), chemical oxygen demand (COD), sodium and other dissolved solids as well as micro-nutrients and heavy metals. Whatever the pollution source may be, soils can act as a sink of heavy metals [13] but three main kinds of ecological risks [19] are associated to this fact:

- the loss of productivity in the soil compartment,
- the pollution of ground-water due to metal leaching, and
- the accumulation of pollutants in the food-chain, with effects on vegetation and animals, including humans


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In India, the abundance of soils with low organic matter content, favours the use of industrial wastewaters containing organic matter as an organic amendment and nutrient supply to soil. Although the benefits of wastewater use in irrigation are numerous but precautions should be taken to avoid short and long-term environmental risks related. Earlier studies have shown that the effect of an industrial effluent vary from crop to crop [10]. So it is essential to study the effect of industrial effluents on individual crops before their use in agricultural fields. Sorghum is a kharif crop which was sown in 9.49 million hectors in 2004 in India, but different cultivars are used in different regions depending upon soil type, local climate and irrigation facilities. Sorghum (*Sorghum vulgare* Pers.) is indigenous to Africa, and many of today's varieties originated on that continent. Sorghum was also grown in India before recorded history and in Assyria as early as 700 BC. Sorghum is a common name for corn-like grasses. It is a dry-land Indian crop most frequently grown as staples in central and western India. Sorghum is mainly grown where rainfall distribution ranges from 10-20 mm per month at least for 3 to 4 months of the south-westerly monsoon or is still more abundant. Farmers grow this crop as fodder for animals in Haryana state of India. A field survey showed that farmers use industrial waste waters for the irrigation of sorghum crop with the intention that the grain of sorghum are not used by human so even industrial wastewaters can be used for its irrigation. Forage sorghum grows 2 to 3 m tall, produces more dry matter tonnage than grain sorghum, is coarse stemmed and used for silage for livestock. Sorghums are best suited to warm, fertile soils; cool, wet soils limit their growth. In the present investigation the impact of textile industry effluent in various concentrations (0, 6.25, 12.5, 25, 50, 75, 100%) on seed germination (%), delay index, shoot length, root length, dry weight and chlorophyll content of two varieties of sorghum viz. *Pioneer jowar* and *Desi jowar* has been explored.

## Review of literature

The volume of wastewater generated from pulp and paper mill varies from 227-455 litres per kg of paper produced. The value of spent wash generated during the process of alcohol production varied from 15-20 litre per litre of alcohol produced, on the other hand, the wastewater generated by a dairy industries being 2-8 litres per litres of milk processed. It was noticed that land application of sewage wastewater has become a common practice in many countries. The reuse of wastewater for irrigation in agriculture is one of the oldest forms of water reclamation. Awareness of this resource is not apparent in the Middle East and North African countries [16]. Due to diversity in industrial growth there is a considerable variation in the quantity and quality of wastewater generated from industry to industry. Management of such effluents to avoid damage to the environment warrants urgent attention. The various approaches [12] relevant to wastewater management under Indian conditions could be

(1) Conservation of various resources, viz., energy, raw materials and water.
(2) Adoption of more efficient processes of manufacturing for better resource utilization.
(3) Water use minimization strategies
(4) Reduction in quantity and quality of wastewater requiring treatment

Laboratory scale experiments were conducted [10] to study the effect of different concentrations (0, 6.25, 12.5, 25, 50, 75, and 100% ) of textile effluents (untreated and


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treated) on seed germination(%), delay index, plant shoot length and root length, plant biomass, chlorophyll content and carotenoid of three different cultivars of wheat. The textile effluent did not show any inhibitory effect on seed germination at low concentration (6.25%). Seeds germinated in 100% effluents but did not survive for longer period. Differential responses of wheat cultivars to effluent treatment were noted. PBW-373 was most sensitive followed by WH-147 and PBW-343. The delay index showed variation for wheat cultivars as well as for effluent concentration. The better growth of all the cultivars at 6.25% effluent concentration may be due to the growth promoting effect of nitrogen and other mineral elements present in the effluent [20, 21].

The germination of kidney bean (*Phaseolus aureus*) and lady's finger (*Abelmoschus esculentus*) seeds were affected adversely when 75 and 100% concentrations of the textile effluent were used as compared to control (water). But there was no effect up to 50% effluent concentration [14]. Where as, Bengal gram (*Cicer arietinum*) seeds' germination was adversely affected even as low as 5% textile effluent concentration [6]. But unlike above said crops, 50% diluted textile effluent increased the seed germination, total sugars, starch, reducing sugars, and chlorophyll than control (distilled water) of groundnut seedlings. These studies showed that the effect of an industrial effluent vary from crop to crop. So it is essential to study the effect of industrial effluents on individual crops before their disposal in agricultural fields.

Saomashekar et al. [22] reported the effect of paper, automobile, textile and food industry wastewaters on soil and seedling growth. The wastewaters of all mills were alkaline in nature with variable concentrations of different chemical species. Application of untreated effluents altered the physicochemical properties of the soil and rate of seed germination was also lower than control. However, the application of diluted effluents was favourable to the seed germination and seedling growth.

The distillery effluents contain various organic and inorganic nutrients and may have a beneficial effect on the crop yield [15, 18]. Ramana et al. [17] reported the effect of different concentrations (0, 5, 10, 15, 25, 50, 75% and undiluted) of distillery effluent (raw spend wash) on seed germination (%), speed of germination, peak value and germination value of some vegetable crops, viz., tomato, chilli, bottle gourd, cucumber and onion. It was concluded that the effect of the distillery effluent is crop-specific and due care should be taken before using the effluent for pre-sowing irrigation purposes. The distillery effluent did not showed any inhibitory effect on seed germination of low concentration expect in tomato. Irrespective of the crop species, at highest concentration (75% and undiluted) complete failure was observed for germination. Based on the tolerance the crops were arranged in order: cucumber > capsicum > onion > bottle gourd > tomato.

Srivastava and Sahai [23] studied the impact of distillery effluents in various concentrations (1, 2.5, 5, 10, 25, 50, 75 and 100%) on the seed germination, speed of germination index, growth, leaf area, biomass, net primary productivity, pigment content, reproduction capacity, seed output, seed weight, seed densities and seed protein content of C*icer arietinum*. It was concluded that very high BOD load and the presence of excessive concentration of soluble salt could be responsible for the toxicity of the effluents. The effluent at up to 5% concentration was, however, beneficial for the overall growth parameter and its use as a liquid fertilizer has been suggested.

Kulkarni et al. [11] classified spent wash as a dilute liquid organic fertilizer, with high potassium content with nitrogen mostly in colloidal forms, which behaves as a "slow release fertilizer" better than most of the inorganic nitrogen sources.


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Dongale and Savant [7] found a significantly higher yield of sugarcane and increase in available N content of soil (300 kg N per ha) through applied spent wash and also thought that spent wash was a good source of potassium for sorghum.

Addition of tannery effluent caused deflocculation of soil particles and increase in the N, P and K levels of soil. Similarly, deleterious effects, such as increase in pH, sodicity and EC in soils due to use of textile effluent have been reviewed by Chhonkar et al. [5]. But the adverse effects of these could be reduced by diluting the effluent. Salinization and alkalization of groundwater due to application of these effluents are also reported.

Ahmad et al [1] studied the response of sugarcane to treated wastewater of oil refinery. The sugarcane growth was better when irrigated with treated wastewater of oil refinery than control (groundwater). The soil receiving wastewater did not show any changes in physicochemical characteristics. The soil accumulated all the heavy metals but the sugarcane accumulated Ni, Pb and Zn only whose values were much lesser then the permissible limits.

Bhati and Singh [4] studied the growth and mineral accumulation in *Eucalyptus camaldulensis* seedlings irrigated with mixed industrial effluent. Addition of textile/municipal effluent increased the survival time for 2 to 3 months. Mixing of effluent was useful in tree irrigation to increase biomass productivity.

Gulfraz et al [8] evaluated the suitability of different industrial effluents (textile mill, oil refinery, soap and detergent mill, hydrogenated oil mill, and rubber industry) for irrigation purposes in wheat crop. The germination of wheat seeds was most affected by textile mill wastewater followed by soap and detergent, oil refinery, hydrogenated oil and rubber industry wastewater. It was concluded that wastewaters should not be discharged in agricultural crops, water stream etc. It was also recommended that industries should install wastewater treatment plants to protect the crops.

## Materials and methods

The effect of textile effluents was studied on two varieties of sorghum (*Sorghum vulgare* Pers.) namely *Pioneer jowar* and *Desi jowar*. Both the cultivars have genetic purity of 95%. The seeds were procured from the certified local seed supplier. The textile effluents (untreated and treated) used in the present study were collected in pre-cleaned containers from a textile mill located near Hisar (Haryana), India. Various physicochemical characteristics of both the effluents were analyzed using standard methods [2]. The effluents were stored at $4^{o}C$ during storage period to avoid any change in its characteristics.

### *Experimental design*

For germination tests, 10 seeds of each sorghum cultivar were placed in sterilized glass Petri dishes of uniform size lined with two filter paper discs. These filter discs were then moistened with 5 ml of distilled water for control and with the same quantity of various concentrations of the textile effluent (6.25, 12.5, 25.0, 50.0, 75.0 and 100%) in distilled water. The Petri dishes were incubated at $30\pm1^{o}C$ in an incubator. Germination was recorded daily at a fixed hour, and the emergence of the radicle was


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taken as a criterion of germination. All the experiments were carried out in triplicate and the results were averaged.

Germination time, defined as the time taken for 60% germination was worked out for studied sorghum cultivars under different effluent concentrations.

Delay index (DI), a normalized parameter, was calculated to compare the performance of different sorghum cultivars under different effluent concentrations as given below.

$$DI = \frac{X}{Y} \qquad \text{(Eq.1)}$$

Where
DI = Delay Index
X= delay in germination time over control (no effluent) and
Y= germination time for control.

For observing seedling growth, five 7 days old seedlings were picked from each of the sets, and the length of the root and shoot were recorded. Plants at the termination of experiment were collected, and their roots and stems along with leaves were separated and dried at 65$^{O}$C in a hot- air oven for 24h. Their dry weights were recorded.

***Table 1.*** *Physicochemical characteristics of textile effluents*

| Parameter | Untreated effluent | Treated effluent |
|---|---|---|
| Colour | Brownish black | Muddy grey |
| pH | 9.9 | 8.2 |
| EC (mmho $cm^{-1}$) | 8.13 | 7.34 |
| Specific gravity | 1.01 | 0.99 |
| Suspended solids ($mgL^{-1}$) | 210 | 128 |
| Total Solids ($mgL^{-1}$) | 7333 | 6786 |
| Total alkalinity (as $CaCO_3$, $mgL^{-1}$) | 946 | 792 |
| Dissolved oxygen ($mgL^{-1}$) | nil | nil |
| BOD ($mgL^{-1}$) | 1626 | 496 |
| COD ($mgL^{-1}$) | 2190 | 960 |
| Total nitrogen (as N) ($mgL^{-1}$) | 246 | 238 |
| Sodium ($mgL^{-1}$) | 186 | 142 |
| Potassium ($mgL^{-1}$) | 9 | 7 |
| Calcium ($mgL^{-1}$) | 318 | 267 |
| Chloride ($mgL^{-1}$) | 860 | 692 |
| Sulphate ($mgL^{-1}$) | 381 | 326 |
| Fluoride ($mgL^{-1}$) | nil | nil |
| Phosphate-P ($mgL^{-1}$) | 18 | 14 |


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*Pot culture experiment*

Pots of 15 cm (diameter) x 14 cm (height) size were filled with equal amounts of sandy loam soil of medium fertility and 10 seeds Pioneer cultivar of sorghum were sown in each pot. The pots were irrigated with selected concentrations (6.25, 12.5, 25, 50, 75 and 100%) of the textile effluents. For each treatment, 100ml of each of these was applied to the respective pot at 5-day interval, throughout the study period. Each treatment had three replications. A control set, irrigated with distilled water was also maintained for comparison. After germination seeds were thinned to five seedlings per pot in all the pots.

The chlorophyll and carotenoid content of the plants were measured. The chlorophyll content was estimated by extracting fresh leaves with 80% acetone and after centrifugation at 8000 rpm for 20min, measuring the colour intensity of the extract at 445, 645 and 663 nm. The formulae of Arnon [3] were used to calculate the *chlorophyll a* and *chlorophyll b* contents and that of Ikan [9] for the carotenoid content.

The data in this study were analyzed using the SPSS package, and all the values are presented as the mean± SE. Student *t*-tests were used to compare the nutritional quality of vermicomposts and fecundity data between the control (cow dung) and other feed wastes. The probability levels used for statistical significance were $P < 0.05$ for the tests.

## Results and discussion

The physicochemical characteristics of untreated and treated forms of the effluent are shown in Table 1. Untreated effluent was brownish black in colour, deficit in dissolved oxygen, rich in total solids, total alkalinity, BOD and COD with considerable amounts of total nitrogen, phosphate, chlorides, sulphates, sodium and calcium. The potassium content was negligible.

Treated effluent was muddy grey in colour. The magnitude of analyzed parameters was lower for treated effluent than untreated effluent (Table 1). The data further showed that suspended solids and BOD content of the studied effluents exceeded the prescribed Indian disposal standards (100 mg $L^{-1}$ and 150 mg $L^{-1}$ respectively).

At lower concentration, the textile effluent (untreated and treated) did not inhibit seed germination in both the varieties. In *Pioneer jowar* 100% seed germination was observed at 6.25% effluent concentration after 120h in both the effluents. Whereas *Desi jowar* seed germination was 100% in untreated effluent and 96.7±5.8% in treated effluent at 6.25% effluent concentration. The germination was inhibited in both the varieties when effluent concentrations exceeded 12.5% in irrigation water. Minimum seed germination was 76.7±5.8% and 83.3±5.8% in *Pioneer jowar* with untreated and treated effluent, and 63.3±5.8 and 80±0.00 in *Desi jowar.* It is evident from the Table 2 that in case of treated effluent the inhibitory effect in *Pioneer jowar* started at 50% effluent concentration onward. Whereas in *Desi jowar* inhibitory effect appeared at lower concentration (6.25%) Table 2. It is observed that germination of *Pioneer jowar* was least affected by both the effluent and showed 100% germination in treated effluent at 6.25%, 12.5% and 25% concentrations. Seed germination (%) of *Desi jowar* was


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inhibited even at 6.25% effluent concentration. So it appears seed germination of *Desi jowar* is more sensitive to effluent irrigation among both the tested varieties.

There was no effect of increasing effluent concentration on delay index in both the varieties up to 50% effluent concentration. The *Pioneer jowar* had a greater value of delay index in both the effluent (UTF and TF) at >50% concentration (Table 3). Whereas in *Desi jowar* the identical delay index value was at 75% concentration (UTF) and at 100% concentration in (TF). The order of delay index among the two verities of sorghum followed this trend: Pioneer (UTF) = Pioneer (TF) < Desi (UTF) < Desi (TF).

***Table 2.*** *Effect of textile effluents on germination (%) of different sorghum cultivars (after 120h) [n= 3, mean± SE]*

| **Effluent** | **Untreated effluent** | | **Treated effluent** | |
|---|---|---|---|---|
| **Conc. (%)** | **Pioneer jowar** | **Desi jowar** | **Pioneer jowar** | **Desi jowar** |
| 0(DW)* | 100± 0.00a | 96.7± 5.8a | 100± 0.00a | 100± 0.00a |
| 6.25 | 100± 0.00a | 100± 0.00a | 100± 0.00a | 96.7± 5.8a |
| 12.5 | 86.7± 5.8b | 96.7± 5.8 a | 100± 0.00a | 96.7± 5.8a |
| 25.0 | 83.3±5.8b | 86.7± 5.8 b | 100± 0.00a | 93.3± 5.8a |
| 50.0 | 76.7± 5.8c | 83.3± 5.8 b | 90± 10.0a | 83.3± 5.8b |
| 75.0 | 76.7± 5.8c | 73.3± 5.8 c | 86.7± 5.8b | 80± 0.00b |
| 100 | 76.7± 5.8c | 63.3± 5.8d | 83.3± 5.8b | 80± 0.00b |

Values followed by same letters in a column are not significantly different ($p \leq 0.05$)

***Table 3.*** *Effect of textile effluent concentrations on delay index (DI) of different sorghum cultivars*

| **Effluent** | **Pioneer Jowar** | | **Desi jowar** | |
|---|---|---|---|---|
| **Conc. (%)** | **UTF*** | **TF**** | **UTF*** | **TF**** |
| 6.25 | 0 | 0 | 0 | 0 |
| 12.50 | 0 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 |
| 50 | 0.50 | 0.50 | 0.25 | 0.25 |
| 75 | 0.50 | 0.50 | 0.50 | 0.25 |
| 100 | 0.50 | 0.50 | 0.50 | 0.50 |

UTF* = Untreated effluent; TF** = Treated effluent

The greatest effect on shoot and root lengths was observed in untreated effluent concentrations (Table 4). There was a decrease at 50, 75 and 100% effluent concentrations in shoot and root lengths of both the tested varieties in untreated as well as treated effluent at all the studied effluent concentrations. The effect was more pronounced for untreated effluent. Shoot length of *Pioneer jowar* in untreated effluent was only 1.3±0.31 cm in 100% concentration which is 9.2 times lower than control (11.9±0.49 cm) (Table 4). For Desi *jowar* shoots emerged in 100% untreated effluent but could not survive. The shoot length of Desi *jowar* in 75% untreated effluent concentration was 1.2±0.25 cm which is 7.4 times lower than control. The roots of both


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the varieties could not survive in 100% untreated effluent. The effect of untreated effluent was more on Desi *jowar* than Pioneer *jowar*. The deleterious effects were more pronounced at 50, 75 and 100% effluent concentrations.

In treated effluent, the effect on shoot and root length of Pioneer *jowar* was lesser. Shoot length of Pioneer *jowar* in 100% treated effluent was 6.1±0.95 cm which is 2 times lower than control (11.9±0.49 cm) (Table 4). The root length of Pioneer *jowar* in 100% treated effluent was 11.2±2.66 cm which is 1.78 times lower than control (19.9±1.68 cm). For Desi *jowar* shoot length was only 1.9±0.40 cm in 100% treated effluent which is 4.7 times lower than control (8.9±0.44 cm) (Table 4). Root length of Desi *jowar* was 2.2±0.25 cm which is 5.13 times lower than control (11.3±1.56 cm). The results also indicated that the treated effluent had lesser deleterious effects than untreated effluent on sorghum.

***Table 4.*** *Effect of textile effluent on shoot and root length of sorghum cultivars (after 7 days) (in cm) [n= 3, mean± SE]*

| **Effluent** | **Pioneer jowar** | | **Desi jowar** | |
|---|---|---|---|---|
| **Conc. (%)** | **SL*** | **RL**** | **SL*** | **RL**** |
| *Untreated effluent* | | | | |
| 0 (DW) *** | 11.9± 0.49a | 19.9± 1.68a | 8.9± 0.44a | 11.3± 1.56a |
| 6.25 | 10.4± 0.91a | 14.0± 1.39b | 6.9± 1.77b | 11.3± 1.71a |
| 12.5 | 9.0± 1.34ab | 11.3± 2.31b | 7.3± 0.97b | 10.8± 2.08a |
| 25.0 | 7.5± 1.10b | 7.87± 0.65c | 1.9± 0.81c | 3.9± 1.17b |
| 50.0 | 5.6± 0.79c | 3.87± 1.56d | 1.5± 0.50c | 3.7± 1.56b |
| 75.0 | 2.1± 0.45d | 2.40± 0.70e | 1.2± 0.25c | 3.8± 0.64b |
| 100 | 1.3± 0.31e | 0.00± 0.00f | 0.0± 0.00d | 0.0± 0.00c |
| T*reated effluent* | | | | |
| 0 (DW) *** | 11.9± 0.49a | 19.9± 1.68a | 8.9± 0.44a | 11.3± 1.56a |
| 6.25 | 11.5± 3.12a | 13.9± 2.50b | 9.0± 0.89a | 17.6± 0.75b |
| 12.5 | 9.1± 1.80a | 13.6± 2.06b | 8.3± 0.28a | 15.5± 0.6bc |
| 25.0 | 8.0± 1.30b | 14.1± 3.49b | 7.0± 1.72b | 14.3± 1.20c |
| 50.0 | 6.8± 2.08c | 13.8± 0.58b | 4.6± 0.65c | 7.9± 1.40d |
| 75.0 | 7.2± 2.00c | 12.2± 0.90b | 3.4± 0.20c | 5.1± 0.66e |
| 100 | 6.1± 0.95c | 11.2± 2.66b | 1.9± 0.40d | 2.2± 0.25f |

SL*= Shoot length, RL** = Root length, (DW) *** = Distilled water
Values followed by same letters in a column are not significantly different ($p \leq 0.05$)

The dry weight of shoots and roots of sorghum also exhibited the similar trend (Table 5). The dry weight of Pioneer *jowar* shoot in untreated effluent was 0.73±0.20 mg per plant at 100% effluent concentration which is 30.7 times lower than control plants (22.4±3.80 mg per plant).

Root biomass of Pioneer *jowar* was 1.1±0.15 mg per plant which is 9.4 times lower than control (10.3±0.20 mg per plant) (Table 5). Effect of untreated effluent was more pronounced at 75 and 100% concentrations for shoot dry weight, where as effect was more pronounced at 50, 75 and 100% concentrations for root dry weights. The shoots of *Desi jowar* could not survive in 100% untreated effluent. The dry weight of *Desi jowar*


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shoot at 75% untreated effluent concentration was 15±0.20 mg per plant which is 12.5 times lower than control (18.8±0.32 mg per plant). The root dry weight of *Desi jowar* in 75% untreated effluent concentration was 1.7± 0.36 mg per plant which is 4.6 times lower than control (7.9±0.41 mg per plant) (Table 5). The results indicated that dry weights of *Desi jowar* were lesser than *Pioneer Jowar*. The effect of treated effluent was comparatively lesser than untreated effluent. The shoot and root of *Desi Jowar* survived as well as grew in 100% treated effluent. The shoot dry weight of *Desi jowar* in 50% treated effluent was equal to 12.5% dose of untreated effluent (Table 5).

***Table 5.*** *Effect of textile effluent on dry weight (after 7 days) of different sorghum cultivars (mg/plant) [n= 3, mean± SE]*

| **Effluent** | **Pioneer jowar** | | **Desi jowar** | |
|---|---|---|---|---|
| **Conc. (%)** | **Shoot** | **Root** | **Shoot** | **Root** |
| *Untreated effluent* | | | | |
| 0 (DW) *** | 22.4± 3.80a | 10.3± 0.20a | 18.8± 0.32a | 7.9± 0.41a |
| 6.25 | 16.6± 0.65b | 10.1± 1.0a | 17.5± 0.86a | 6.6± 0.80ab |
| 12.5 | 15.8± 0.40b | 11.0± 0.78a | 10.1± 0.36b | 6.1± 0.35b |
| 25.0 | 14.6± 0.35b | 7.1± 0.85b | 6.6± 0.15c | 2.7± 0.43c |
| 50.0 | 14.2± 0.45b | 4.9± 0.56c | 2.8± 0.40d | 1.8± 0.10d |
| 75.0 | 8.6± 0.35c | 3.4± 0.30d | 1.5± 0.20e | 1.7± 0.36e |
| 100 | 0.73± 0.20d | 1.1± 0.15e | 0.0± 0.00f | 0.0± 0.00f |
| T*reated effluent* | | | | |
| 0 (DW) *** | 22.4± 3.80a | 10.3± 0.20a | 18.8± 0.32a | 7.9± 0.41a |
| 6.25 | 18.7± 0.62b | 10.6± 0.31a | 20.7± 2.10a | 9.4± 0.35a |
| 12.5 | 17.9± 0.20b | 9.9± 0.35a | 19.6± 1.38a | 7.5± 0.20a |
| 25.0 | 15.2± 0.40c | 7.5± 0.55b | 17.5± 0.90a | 6.5± 0.25b |
| 50.0 | 14.3± 0.20c | 7.1± 0.76b | 10.3± 0.30b | 3.3± 0.26c |
| 75.0 | 12.2± 0.31d | 6.8± 0.40bc | 9.8± 0.59b | 3.0± 0.10d |
| 100 | 10.3± 0.30d | 6.1± 0.30c | 9.0± 0.75b | 2.1± 0.15e |

Values followed by same letters in a column are not significantly different ($p \leq 0.05$)

The *chlorophyll a* and *chlorophyll b* contents were increased at 6.25% concentration and decreased significantly at higher concentrations by both the effluents. Similar observation has been reported by other co-workers [21] in *Phaselous radiatus* treated with distillery effluent. The inhibitory effect of untreated effluent was more on the pigments than treated effluent. The carotenoid content also increased up to 6.5% effluent concentration and decreased at higher concentrations (Table 6).


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***Table 6.*** *Effect of textile effluent on plant pigments of Pioneer Jowar [after 30 days] (mg $g^{-1}$ fresh weight) [n= 3, mean± SE]*

| Effluent Conc. (%) | Chl. a | Chl. b | Total Chl. | Carotenoid |
|---|---|---|---|---|
| *Untreated Effluent* | | | | |
| 0(DW)* | 0.618 ± 0.084a | 0.274 ± 0.035a | 0.931 ± 0.051a | 1.62 ± 0.063a |
| 6.25 | 0.636 ± 0.075a | 0.271 ± 0.041a | 0.946 ± 0.058a | 1.73 ± 0.095a |
| 12.5 | 0.603 ± 0.064a | 0.235 ± 0.032a | 0.859 ± 0.035a | 1.58 ± 0.084a |
| 25.0 | 0.578 ± 0.067a | 0.217 ± 0.025ab | 0.814 ± 0.037a | 1.35 ± 0.067ab |
| 50.0 | 0.413 ± 0.059b | 0.178 ± 0.012b | 0.612 ± 0.023b | 1.24 ± 0.048b |
| 75.0 | 0.245 ± 0.026c | 0.153 ± 0.017b | 0.362 ± 0.014c | 0.67 ± 0.045c |
| 100 | 0.00 ± 0.00d | 0.00 ± 0.00c | 0.00 ± 0.00d | 0.00 ± 0.00d |
| *Treated effluent* | | | | |
| 0(DW)* | 0.618 ± 0.084a | 0.274 ± 0.035a | 0.931 ± 0.051a | 1.62 ± 0.063a |
| 6.25 | 0.632 ± 0.068a | 0.281 ± 0.032a | 0.987 ± 0.053a | 1.67 ± 0.035a |
| 12.5 | 0.625 ± 0.047a | 0.275 ± 0.031a | 0.973 ± 0.043a | 1.58 ± 0.037a |
| 25.0 | 0.588 ± 0.051a | 0.268 ± 0.027a | 0.958 ± 0.036a | 1.42 ± 0.021a |
| 50.0 | 0.513 ± 0.071a | 0.263 ± 0.024a | 0.917 ± 0.051a | 1.31 ± 0.025a |
| 75.0 | 0.472 ± 0.032b | 0.245 ± 0.018a | 0.818 ± 0.026a | 1.37 ± 0.034a |
| 100 | 0.386 ± 0.035b | 0.236 ± 0.037a | 0.674 ± 0.038b | 1.24 ± 0.051a |

Values followed by same letters in a column are not significantly different ($p \leq 0.05$)

## Conclusion

The physicochemical characteristics of the textile mill wastewater exceeded the prescribed Indian standards. So some more effective treatment is necessary to minimize the pollution effects before the textile industry effluent is discharged on the land. But disposal of these effluents after proper dilution may be a favourable approach. After dilution, the effluent characteristics come within the prescribed disposal limits and pollution load per unit effluent volume is decreased. The better growth of both the cultivars at 6.25% effluent concentration may be due to the growth promoting effect of nitrogen and other mineral elements present in the effluent Differential responses of sorghum cultivars to untreated and treated wastewater were noted. Pioneer Jowar was lesser affected than Desi Jowar by both the wastewaters. The use of wastewater for irrigation may serve as an additional source of water with fertilizing properties after appropriate dilution. Irrigation water quality not only affect the growth of crops, but also have long term effects on soil health, grain quality, fodder quality and health of consumers. So finally it is suggested that long term experiments should be conducted to explore the effect of textile mill wastewater on above suggested aspects before its use for irrigation.


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# EARTHWORM (*EISENIA FETIDA*) BIOASSAY TO ASSESS THE POSSIBLE EFFECTS OF PLATINUM TAILINGS DISPOSAL FACILITIES ON THE ENVIRONMENT ALONG A GRADIENT

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**Abstract.** Platinum mines produce large amounts of inorganic tailings containing high levels of metals which are disposed of on tailings disposal facilities (TDFs) and there is no information available on their possible effects on the surrounding terrestrial environment. The aim of this study was to do an earthworm bioassay of soils along a gradient from a TDF over a period of 28 days in terms of growth, reproduction and metal accumulation. After 28 days the earthworms in the soil collected up to 1 km away from the TDF showed a significant ($P < 0.05$) decrease in bodyweight and in the soil 2–5 km away showed no effect. The earthworms in the soil collected 15 km away from the TDF showed a significant ($P < 0.05$) increase in bodyweight. The mean hatching success of cocoons was significantly ($P < 0.05$) higher in the soils further away from the TDF *viz.* 15 km > 3–5 km > 2–0 km. Cr, Cu, Ni and Zn concentrations in the soils collected in the soils on the platinum TDF (TDF – 15 km in the case of Zn) were higher, while Cd, Co and Pb were lower when compared to screening benchmarks proposed by the U.S. Dept. of Energy. The presence of these metals in a mixture, however, makes it extremely difficult to assess their effects. All of the metals had low bioconcentration factors (BCFs) *viz.* < 0.01 (CR, Ni and Pb), 0.01 (Co), 0.33–0.5 (Cd), 0.01–0.08 (Cu) and 0.18–0.29 (Zn). It can be concluded that platinum mining, with TDFs as source of contamination, has negative effects on the environment but further studies are needed to assess the exact extent of these effects.
**Keywords**: *bioassay; earthworms; Eisenia fetida; metals; platinum mining*

## Introduction

Mining activities in South Africa contribute to more than 70% of the solid waste stream [1] and use an estimated 25 000 ha of land as dumping areas [2]. The country produces 50% of the world's platinum and holds 55% of global reserves, of which most (> 70%) is situated in the North-West Province of South Africa [3]. These areas are therefore inherently associated with the wastes produced by these mines. At one such platinum mine (second largest producer in South Africa) large amounts of inorganic tailings are produced, consisting mainly of sand and silt, which is disposed of on tailings disposal facilities (TDFs). From a previous study done by Van Rensburg and Morgenthal [4] it was evident that these tailings contained high levels of metals compared to the standards proposed by the South African Department of Water Affairs and Forestry [5]. TDFs, therefore pose a range of possible environmental impacts including air, dust and groundwater pollution. At present there is, however, no information available on the possible effects platinum TDFs might have on the surrounding terrestrial environment.

Analytical methods used to measure the levels of contaminants in soils may result in misleading indications of the bioavailability of the contaminants [6, 7]. The use of biological indicators to provide information on the environmental risks of contaminated


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sites has therefore been proposed as a tool in risk assessment by the United States Environmental Protection Agency [USEPA] [8] and the Canadian Government's contaminated sites remediation program [9]. At present there are, however, no guidelines or policies in South Africa regarding levels of contaminants in soil that would not pose environmental risks. In an overview on soil biology and biochemistry in South Africa, Haynes and Graham [10] ascribed this lack in guidelines to the paucity of research done in this field.

Bioassays, using earthworms and soil from contaminated field sites, are recommended [11] for determining the actual toxicity and risk of contaminated soil and have been used in numerous studies [12, 13, etc.] since they provide an index to the bioavailability of contaminants present in these soils. Further, growth and reproduction of the earthworms can provide additional information about the sublethal effects on the worms and potential environmental hazards, since earthworms can be seen as sentinel soil organisms.

The aim of this study was to do a bioassay of soils along a gradient from a TDF to assess the possible impact it has on the surrounding terrestrial environment. This was achieved by collecting soils from the field, determining its metal concentrations and exposing earthworms (*Eisenia fetida*) to it in a 28-day bioassay. Further, to evaluate the changes in biomass and reproduction of the earthworms to assess sublethal effects on them, as well as metal contents of their body tissues.

## Materials and methods

Tests were performed according to guidelines formulated by the Office of Prevention, Pesticides and Toxic Substances [14] a sub directorate of the USEPA.

A random sampling design was used to obtain three composite samples per site (each composite sample consisting of 6 subsamples) of the topsoil (0 – 15 cm) from each of the sites investigated *viz.* 0- (on the toe of the TDF), 1-, 2-, 3-, 4-, 5- and 15 km, the last serving as reference ("control") for the purpose of the study. The collected soils were air dried after collection and sieved (< 2 mm) before it was moistened with distilled water to 60% (by weight) moisture content. The substrate was placed into plastic containers (15 x 10 x 5 cm), covered with a piece of plastic before putting on a perforated lid, and kept in an environmental chamber (20°C) for 48 hours to stabilise, before introducing the earthworms. The pH of the soil was determined in the 1:2 extract with a calibrated pH/conductivity meter (Radiometer PHM 80, Copenhagen).

The breeding stock of *E. fetida* used in this study was obtained from a local worm breeder in the Potchefstroom (North-West Province of South Africa) and maintained on cattle manure at a temperature of 20°C (±1°C). Only mature clitellate worms were used for the purposes of this investigation.

Ten earthworms were placed in each of the three replicate containers filled with 500 g of the prepared soils. Feeding of the worms comprised sprinkling 5 g of dried cattle manure per container per week. Metal concentrations in the control and exposure group substrates were determined prior to the start of the experiment. The biomass of the earthworms was determined at the start of the experiment (day 0) and at the end (day 28) and the moisture content (60 %) and pH (7) of the substrates was monitored every 7 days.

Cocoon viability was determined by harvesting 15 cocoons from each container and placing them in multidishes filled with distilled water. The water in these dishes were


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changed every third day to prevent bacterial growth, which could impact negatively on the results. The number of hatched cocoons was recorded over a period of four weeks.

Mortality was determined by counting the number of dead earthworms. Worms were considered dead if they lacked movement and did not respond to a definite tactile stimulus to the anterior end. Because earthworms tend to disintegrate quickly after death, absent earthworms were considered to have died.

### *Metal analysis*

At termination of the experiment four earthworms per replicate ($n$ = 12) were removed from the substrate. These worms were placed on wet filter paper in Petri dishes for a period of 48 h to allow the depuration of their gut contents. This was done to determine the actual metal content in the body tissues in order to calculate bioconcentration factors (BCFs). After this 48-h period the worms were washed in distilled water, dried on paper towels and killed by freezing. They were individually weighed and frozen (-74°C) in polypropylene vials for metal analysis at a later stage. Three samples per replicate ($n$ = 9) of the substrate were also removed, placed in plastic bags and refrigerated until metal analysis. Worms and soil samples were digested as described by Katz & Jennis [15] and analysed on an Agilent 7500c Inductive coupled plasma mass-spectrometer (ICP-MS).

### *Statistical analysis of data*

The data in this study was analysed by using the SigmaStat® computer software package and all values presented as the mean ± SD (standard deviation). The probability levels used for statistical significance were $P < 0.05$ and parametric or non-parametric tests were used to compare groups.

## Results and discussion

At no stage during the study were any mortality observed and the results regarding changes in bodyweight and mean number of cocoons hatched are summarised in Table 1. On day one (initiation of experiment) there was no significant ($P > 0.05$) difference between the bodyweights of the earthworms introduced into the different soils. After 28 days (termination of experiment) the earthworms in the soil collected up to 1 km away from the TDF showed a significant ($P < 0.05$) decrease in bodyweight and in the soil 2–5 km showed no effect. The earthworms in the soil collected 15 km away from the TDF showed a significant ($P < 0.05$) increase in bodyweight. The mean hatching success of cocoons, which gives a good representation of reproductive success, was significantly ($P < 0.05$) higher in the soils further away from the TDF *viz.* 15 km > 3–5 km > 2–0 km. Reproductive success as indicator was therefore a more sensitive parameter than growth insofar as to assess the effects of platinum mining on the environment.


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***Table 1.*** *Mean bodyweight (g) and percentage weight change of earthworms (n = 30) exposed to soils collected at different distances (in km) from the tailings disposal facility (TDF) at the start (day 1) and end (day 28) of the bioassay, as well as mean number and hatching success of cocoons produced.*

| Site | Day 1 | Day 28 | Weight change (%) | Mean % cocoons hatched (*n* = 15) |
|---|---|---|---|---|
| **TDF** | 0.42±0.07[a] | 0.37±0.05[a*] | – 13.08* | 30.75±3.42[a] |
| **1** | 0.41±0.10[a] | 0.36±0.08[a*] | – 12.67* | 31.29±0.93[a] |
| **2** | 0.44±0.07[a] | 0.40±0.08[a] | – 5.51 | 34.68±1.22[a] |
| **3** | 0.39±0.07[a] | 0.38±0.06[a] | – 2.54 | 54.24±2.52[b] |
| **4** | 0.40±0.08[a] | 0.39±0.08[a] | – 2.09 | 54.21±6.5[b] |
| **5** | 0.42±0.08[a] | 0.41±0.07[a] | – 1.60 | 56.02±4.7[b] |
| **15** | 0.40±0.04[a] | 0.45±0.08[b] | 12.50 | 74.32±1.13[c] |

* significantly different from initial bodyweight ($P < 0.05$)
[a,b,c] Means with the same letter were not significantly different ($P > 0.05$)

The metal concentrations and pH measured in the soils and earthworm body tissues are summarised in Table 2, which includes screening benchmarks (SBs) proposed by the U.S. Dept. of Energy [16] as well as target concentrations (TCs) and intervention concentrations (ICs) proposed by the Dutch Government (VROM) [17]. SBs are the concentrations deemed to be of potential concern to specifically earthworms as target organisms. TCs are the levels of contamination at which there is sustainable soil quality, whilst the ICs indicates concentrations of contaminants that may seriously impair the function of human, plant and animal life. The reason for using benchmarks proposed by Efroymson *et al.* [16] and VROM [17] is that at present no such information is available for South African soils.

Cr, Cu, Ni and Zn concentrations in the soils collected in the soils on the platinum TDF (TDF – 15 km in the case of Zn) were higher than the SBs and the TCs but lower than the ICs. Cr in the sites 1–15 km away from the TDF the measured concentrations were higher than the SB but lower than the TC, while the Cu concentrations were below the SBs and TCs for the same sites. Ni in these sites (1–15 km away from the TDF) was below the SB and IC bit higher than the TC. Cd, Co and Pb were all higher than the TCs but lower than the IC and SBs for all the sites (TDF–15 km). The effects of Cr [18, 19], Cu [20, 21], Ni [22] and Zn [23] on either growth or reproduction of earthworms has been reported on. These reported effects were, however, at higher metal concentrations than found in the present study, but it has to be taken into account that these studies were conducted using single metals as contaminants. Based on previous studies regarding Cd [21, 23, 24], Co [16] and Pb [21, 24] it can be concluded that these metals should not be of concern in the soils on a gradient away from platinum TDF. The presence of these metals in a mixture, however, makes it extremely difficult to assess the effects these metals might have on earthworms since the actual toxicity is determined by the bioavailability of toxicants. The reason for this being that pollutants in mixtures might be additive, antagonistic or synergistic in the effects they have on organisms. Khalil *et al.* [25] e.g. concluded that the presence of Cd, Cu and Zn below $EC_{50}$ concentrations might affect growth negatively, but also stated that there is little agreement in the literature about metal mixture toxicity. All of the metals had low BCFs *viz.* < 0.01 (CR, Ni and Pb), 0.01 (Co), 0.33–0.5 (Cd), 0.01–0.08 (Cu) and 0.18–0.29 (Zn). Since these BCFs were all < 1 it is indicative that the potential for earthworms to


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accumulate it in their body tissues are limited, which could be a consequence of the high pH levels of the soils (Table 2).

***Table 2.*** *Selected metal concentrations (µg $g^{-1}$ unless indicated otherwise) of soils (dry weight, n = 9) and earthworms (E/w) utilised in soils collected at different distances (in km) from the tailings disposal facility (TDF), calculated bioconcentration factors (BCF) of earthworm body tissues (n = 12) after termination of a 28-day bioassay, screening benchmark concentrations (USDE, 1997) as well as target (TC) and intervention concentrations (IC) [VROM, 2000].*

| | | **Cd** | **Co** | **Cr** | **Cu** | **Ni** | **Pb** | **Zn** | **pH** |
|---|---|---|---|---|---|---|---|---|---|
| **USDE** | | **20** | **–** | **0.4** | **50** | **200** | **500** | **200** | |
| **VROM (TC)** | | **0.8** | **9** | **100** | **36** | **35** | **85** | **140** | |
| **VROM (IC)** | | **12** | **240** | **380** | **190** | **210** | **530** | **720** | |
| **Site** | | | | | | | | | |
| **TDF** | Soil | 2.23± 0.28[a] | 220.00± 20.00[a] | 263.33± 11.55[a] | 67.67± 18.18[a] | 210.00± 88.88[a] | 373.33± 41.63[a] | 300.00± 43.59[a] | 8.08± 0.05[A] |
| | E/w | 0.85± 0.41[a] | 2.76± 1.26[a] | 1.62± 0.37[a] | 0.98± 0.54[a] | 0.26± 0.13[a] | 0.25± 0.14[a] | 63.03± 31.98[a] | |
| | BCF | 0.38 | 0.01 | < 0.01 | 0.01 | < 0.01 | < 0.01 | 0.21 | |
| **1** | Soil | 2.53± 1.10[a] | 230.00± 30.00[a] | 69.33± 20.40[b] | 32.33± 1.15[b] | 113.33± 15.28[b] | 446.67± 30.55[a] | 353.33± 30.55[a] | 7.90± 0.13[A] |
| | E/w | 0.98± 0.43[a] | 2.34± 0.73[a] | 0.38± 0.18[b] | 0.94± 0.26[a] | 0.27± 0.12[a] | 0.20± 0.14[a] | 93.75± 35.13[a] | |
| | BCF | 0.39 | 0.01 | < 0.01 | 0.03 | < 0.01 | < 0.01 | 0.27 | |
| **2** | Soil | 2.50± 0.17[a] | 216.67± 20.82[a] | 63.00± 1.00[b] | 18.00± 3.61[c] | 101.00± 8.54[b] | 383.33± 87.37[a] | 343.33± 68.07[a] | 8.01± 0.08[A] |
| | E/w | 0.83± 0.56[a] | 2.74± 1.43[a] | 0.30± 0.03[b] | 0.84± 0.38[a] | 0.31± 0.19[a] | 0.31± 0.16[a] | 85.17± 29.77[a] | |
| | BCF | 0.33 | 0.01 | < 0.01 | 0.05 | < 0.01 | < 0.01 | 0.25 | |
| **3** | Soil | 2.13± 0.40[a] | 160.00± 26.46[b] | 61.33± 12.22[b] | 13.27± 4.24[c] | 80.67± 18.56[b] | 393.33± 40.41[a] | 390.00± 36.06[a] | 8.10± 0.03[A] |
| | E/w | 0.92± 0.78[a] | 2.21± 1.55[a] | 0.31± 0.05 | 0.76± 0.45[a] | 0.27± 0.16[a] | 0.22± 0.09[a] | 69.19± 42.76[a] | |
| | BCF | 0.43 | 0.01 | < 0.01 | 0.06 | < 0.01 | < 0.01 | 0.18 | |
| **4** | Soil | 2.40± 0.87[a] | 290.00± 26.46[b] | 72.00± 8.54[b] | 12.67± 0.58[c] | 99.00± 1.73[b] | 503.33± 56.86[a] | 323.33± 37.86[a] | 8.03± 0.04[A] |
| | E/w | 1.21± 0.54[a] | 3.28± 0.83[a] | 0.39± 0.13[b] | 0.99± 0.29[a] | 0.35± 0.16[a] | 0.26± 0.13[a] | 88.08± 13.98[a] | |
| | BCF | 0.50 | 0.01 | < 0.01 | 0.08 | < 0.01 | < 0.01 | 0.27 | |
| **5** | Soil | 2.17± 0.23[a] | 203.33± 23.09[a] | 66.67± 10.21[b] | 17.67± 1.15[c] | 90.67± 9.02[b] | 403.33± 20.82[a] | 306.67± 30.55[a] | 7.89± 0.08[A] |
| | E/w | 1.02± 0.67[a] | 3.35± 0.86[a] | 0.29± 0.02[b] | 0.82± 0.20[a] | 0.32± 0.14[a] | 0.19± 0.07[a] | 89.73± 16.09[a] | |
| | BCF | 0.47 | 0.02 | < 0.01 | 0.05 | < 0.01 | < 0.01 | 0.29 | |
| **15** | Soil | 2.11± 0.93[a] | 176.67± 11.55[b] | 70.00± 3.46[b] | 10.30± 0.61[c] | 73.33± 0.58[c] | 400.00± 30.00[a] | 360.00± 70.00[a] | 8.02± 0.11[A] |
| | E/w | 1.08± 0.45[a] | 3.22± 0.74[a] | 0.38± 0.01[b] | 0.73± 0.25[a] | 0.19± 0.09[a] | 0.21± 0.09[a] | 91.83± 16.36[a] | |
| | BCF | 0.43 | 0.02 | < 0.01 | 0.07 | < 0.01 | < 0.01 | 0.26 | |

[a,b,c] Means with the same letter were not significantly different (P > 0.05)

BCF = $^{[e/w]}/_{[substrate]}$

< Denotes concentrations that were below the detection limit.


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Bioassays of soils collected from the field do, however, indicate how these metal mixtures, in conjunction with soil properties (e.g. pH), affect soil organisms and are environmentally more "realistic" than single toxicant studies.

## Conclusions

Although most of the metals present in the soils studied were higher than the screening benchmarks [16] it is, however, extremely difficult to interpret the effects of these metals due to the fact that they are present in a mixture. Having stated this, the fact remains that it is possible to compare the relative toxicity of the soil, or rather the effects of the platinum TDF, along an increasing gradient on the basis of effects on earthworm mortality, growth and reproduction. Based on the data regarding growth it can therefore be concluded that TDFs of platinum mining only has a negative effect on earthworms up to 1 km and up to 5 km if reproduction is used as effect parameter. These effects on growth and reproduction can be attributed to the presence of metals in these soils, especially Cr, Cu and Ni which were all found to be decreasing in concentration in a gradient away from the TDF.

The fact that the soils collected were from an industrially polluted area and that most metal concentrations were below ICs even on the TDF suggests that the impact of platinum mining on the environment is not too severe. Growth and reproduction data on *E. fetida,* however, suggested otherwise. In a study to biologically assess contaminated land using earthworms, Hankard *et al.* [12] found that effects manifested a cellular level of the worms despite the fact that metal concentration were below the ICs. Since the dynamics of metal mixtures are not fully understood yet, it should always be considered to do bioassays rather than base environmental impacts on chemical data alone. From the results presented it can therefore be concluded that platinum mining, with TDFs as source of contamination, has negative effects on the environment for up to 5 km. Further studies between 5–15 km are, however, needed to assess the exact negative effects and it can further be concluded that the effects of platinum mining on the terrestrial environment is less than 15 km away from TDFs.

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# EFFECT OF PYRIDINE AND FORMALDEHYDE ON A MACROPHYTE (*LEMNA MINOR* L.) AND A SLUDGE WORM (*TUBIFEX TUBIFEX* MÜLLER) IN FRESH WATER MICROCOSMS

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**Abstract.** Pyridine, a suspected genotoxic and formaldehyde, a potent carcinogen, are present in several industrial wastewater including commercial solvent, resin and pesticide manufacturing industries. The extent of pyridine and formaldehyde toxicity in aquatic organisms is unknown. Therefore, in present study the toxicity of pyridine and formaldehyde were investigated in artificial aquatic ecosystem (microcosm) using two freshwater organisms of different trophic levels i.e. a common duckweed; Lemna minor L. and an oligochaete; Tubifex tubifex Müller. In microcosm, formaldehyde was found to be more toxic to chlorophyll (21 days IC50 = 0.08±0.02 mgL-1), protein (21 days IC50 = 0.15±0.02 mgL-1) and biomass (21 days IC50 = 0.18±0.02 mgL-1) contents of L. minor than pyridine. In addition, pyridine was found growth supportive to L. minor at concentration of 0.1-0.3 mgL-1. Formaldehyde was found more toxic than pyridine for T. tubifex. Lethal concentrations for formaldehyde were 0.08±0.004 (LC10–21 days), 0.2±0.03 (LC25–21 days) and 0.39±0.05 mgL-1 (LC50–21 days). For pyridine, lethal concentrations were 0.85±0.08 (LC10-21 days), 2.14±0.2 (LC25-21days) and 4.27±0.3 mgL-1 (LC50-21days). The concentration and time dependent decrease in growth rate, soluble protein and glycogen content was observed. To overcome the stress situation, high-energy requirement of worms lead to protein and glycogen catabolism. The results of present study might be an important consideration while assessing the hazards of materials to aquatic organisms or when deriving water quality criteria for aquatic organisms.
**Keywords.** *Lemna minor L.; Tubifex tubifex Müller; formaldehyde; pyridine; toxicity; microcosms*

## Introduction

Increasing industrialization and continuous production of new chemicals has necessitated the advancement of methods for monitoring and treatment to evaluate their damaging effect on the environment. Low acute toxicities are detected in effluents that are discharged from efficient secondary treatment plants [15]. However, such effluents can still include harmful substances that can cause long-term effects by being, for example, genotoxic, hormonally active or bio-accumulative. Many of these substances appear at low concentrations and are of unknown chemical character, which not only makes their chemical analysis time consuming but also requires high tech methods for analysis [35]. For these reasons the use of eco-toxicity tests in effluent monitoring would be useful. In the field of environmental risk assessment, the microcosm is a valuable tool that can be used for evaluation of pure substances as well as whole effluent toxicity (WET) in laboratory. Although smaller and less complex than real


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world freshwater ecosystems, microcosms provide an opportunity to perform ecosystem level research in replicate tests systems under conditions that are manageable in terms of costs and logistics [13].

Pyridine, a *N*-heterocyclic compound, is widely used as solvent in dyes, explosives, pharmaceuticals, pesticides and agro based industries [21]. Pyridine is found in wastewater from these industries that cause human health hazards [44]. Formaldehyde is found in wastewater from resin manufacturers [20] and petrochemical plants [39]. Formaldehyde is also used as an active ingredient in preservatives and disinfectants. Its disposal after use may impair sewage treatment plant performance due to its antimicrobial effects. The demonstration of its carcinogenicity in laboratory animals led to a heightened concern that formaldehyde may present a similar carcinogenic risk to humans [42].

The extent of pyridine and formaldehyde toxicity in aquatic organisms is not well documented. Thus, our purpose of investigation was to evaluate the toxicity of pyridine and formaldehyde to aquatic organisms. We chose two aquatic test models of two different trophic levels, *Lemna minor* L., a higher plant, and a bottom dweller sludge-worm, *Tubifex tubifex* Müller.

Common duckweed, *Lemna minor* L. (Lemnaceae) is a worldwide species and is available throughout the year for use in ecotoxicological research. In addition, the smaller size, simple structure, asexual reproduction and short generation time makes this plant a very suitable candidate for laboratory testing [2, 31]. Parameters, such as total chlorophyll, total protein and biomass, used in the toxicity assays were examined. *Tubifex tubifex* Müller is a small freshwater endobenthic oligochaete worm that lives in the shallows of freshwater ponds, lakes and marshes containing decomposing organic materials. Because of its wide distribution and culture ease *T. tubifex* have been proposed as a test organism for ecotoxicological studies [12, 30]. Parameters, such as lethal concentrations ($LC_{10}$, $LC_{25}$ and $LC_{50}$), growth rate, total protein and glycogen content were studied to determine the toxicity impact of pyridine and formaldehyde.

## Materials and methods

### *Sediment collection and characterization*

Samples were collected from ITRC (Gheru campus), Lucknow ($26^0$ 55'N; $80^0$ 59'E), India on November 2, 2004. General characteristics (sediment particles size, total organic carbon, bulk density, mineral density, water content and porosity) of soil sample were characterized by standard methods [4]. Each bulk sample was homogenized and sieved through a 1 mm mesh to remove debris of plants and microfauna. Dechlorinated tap water (*Table 1.*) was added to the bulk of soil samples. The soil slurries obtained were used to prepare the microcosm.

### *Chemicals*

All chemicals used were of analytical grade and purchased from Sigma-Aldrich, Banglore, India. Manufacturers provided 980 and 1080 $mgmL^{-1}$ strength of pyridine and formaldehyde, respectively. Ten milliliter of pyridine and formaldehyde dissolved in 980 and 1080 mL distilled water provided strength of 10 $mgL^{-1}$ used as stock solution. Zero, 3, 6, 9, 12, 15, 18, 21, 24 and 27 mL of stock solution was dissolved in 300 mL


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soil slurry for strength of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 $mgL^{-1}$ pyridine and formaldehyde.

***Table 1.*** *Physico-chemical characteristics of soil and dilution water (tap water)*

| **Sediment particles size fraction (%) and other characteristics** | | | **Dilution water ($mgL^{-1}$)** | |
|---|---|---|---|---|
| Particles size | <15 µm | 46.4 ±3 | Potassium | 4.10 ±0.06 |
| | 15-30µm | 27.7 ±2 | Sodium | 35.25 ±0.7 |
| | 30-63µm | 20.0 ±0.8 | Sulphate | 8.55 ±0.08 |
| | 63-125µm | 4.4 ±0.07 | Chloride | 5.0 ±0.08 |
| | 125-250µm | 1.4 ±0.02 | Ammonium | 0.35 ±0.004 |
| | 250-500µm | 0.1 ±0.003 | Total Solids | 350 ±31 |
| | >500µm | - | Total Dissolved Solids | 325 ±23 |
| Organic carbon | | 16.5 ±0.07 | Total Suspended Solids | 25 ±1 |
| Bulk density ($gcm^{-3}$) | | 0.08 ±0.004 | pH | 8.27 ±0.3 |
| Mineral density (gcm-3) | | 0.03 ±0.001 | Dissolved Oxygen | 1.10 ±0.05 |
| Water content | | 90.2 ±4 | Calcium | 15.25 ±0.2 |
| Porosity | | 0.74 ±0.04 | Total Organic Contents | 0.75 ±0.04 |
| *Heavy metals* | | | | |
| Cd, Ni Pb, Cr, Zn and Cu | | ND* | Cd, Ni Pb, Cr, Zn and Cu | ND |
| Fe | | 0.4 ±0.02 | Fe | 0.2 ±0.04 |

*ND- not detected; all values are mean (n=3) ±S.D.

### *Microcosm preparation*

A total of 120 microcosms were prepared for pyridine and formaldehyde. Individual microcosms were prepared in 1 L polycarbonate Imhoff settling cones, plugged at the bottom with No. 4 silicon rubber stoppers. Each cone was filled with 150 mL of the soil slurry, the sides were then rinsed down and the final volume in each cone was brought upto 300 mL with desired quantity of stock pyridine and formaldehyde.

### *Lemna minor*

The duckweed culture was collected from a local water body. Water sample were taken and analyzed to confirm that the water body was not laden with pollutants like heavy metals (Cr, Zn, Cu, Cd, Ni and Pb) and organic chemicals (formaldehyde and pyridine, particularly). The stock was maintained in the laboratory in a plastic bucket with a 20-L capacity, according to the procedure reported by Chandra and Singh [11]. The stock solution of culture media of *Lemna minor* was prepared following by OECD guidelines [33].

Twenty four hours before the treatment was initiated duckweed samples were selected for the experiment. A spoonful of duckweed from the stock was placed in a small tray containing cold tap water. Hundred experimental specimens having two fronds of approximately equal size per colony were selected from this tray and transferred to a second tray containing only distilled water. Frond numbers were counted manually with the aid of magnifying glass. Change in fronds e.g. chlorosis (yellowing of frond tissue) and necrosis (white dead frond tissue) was visually observed. Total chlorophyll content was determined on a fresh-weight ($mgg^{-1}$) basis according to standard method [43] on a spectrophotometer (GBC Cintra-40, Australia)


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at absorbance of 645 and 663 nm using a quartz glass cuvette with 1 cm thickness. Protein content ($mgg^{-1}$) was spectrophotometrically determined by bicinchoninic acid (BCA) method [10] at absorbance of 562 nm. Biomass was determined by the dry weight method [2] in $mgg^{-1}$. For convenience, all data in $mgg^{-1}$ were converted in %. Data were statistically analyzed by an overall one-way analysis of variance (ANOVA) and when differences observed were significant, the mean were compared by Tukey-Kramer Multiple Comparison Test. $IC_{50}$ (concentration of compound to cause 50% reduction of biological processes) values were graphically estimated.

*Tubifex tubifex*

*Tubifex tubifex* worms were obtained from a local fish food supplier. The worms were reared at low density (100- 250 individuals) in a 2-L aquarium containing natural sediment and tap water (*Table 1.*) To avoid accumulation of ammonia, the water was replaced weekly and the worms fed with spinach once a week. The aquarium was placed in a growth chamber in which temperature was $21\pm1^0$C and the lighting was 12 h dim light/ 12 h darkness. For luxurious growth, dissolved oxygen (DO) was maintained at 60% saturation in static cultures [35]. We maintained DO levels by replacing culture water in every 7 days, adding fresh dechlorinated tap water to accommodate evaporation, generous aeration and regulating food. The worms were held for 6 months before experiment. The worms were removed from the sediment 24 h before use and stored in petri dishes on damp filter paper (in the dark at $14\pm1^0$C) to void gut contents. The selection criteria were equal size and color of body.

Tests were conducted to determine the lethal concentrations ($LC_{10}$, $LC_{25}$ and $LC_{50}$) of pyridine and formaldehyde for worms under laboratory conditions. Fifty worms were introduced in to each microcosm and placed into an incubation chamber ($21\pm1^0$C, and 12:12 h photoperiod). Three replicates without test compounds were used as control. After 7, 14 and 21 days, dead worms were counted and $LC_{10}$, $LC_{25}$ and $LC_{50}$ values were determined graphically according to Ammon [1]. LC values from three experiments were averaged and mean ±standard deviations are presented.

After 7, 14 and 21 days of exposure the weight of *T. tubifex* was compared to that of control untreated worms. The growth rates were determined using the equation of Martin [26].

Relative growth rate = $ln\ Wt/Wo\ x\ 100$

Where, *Wo* is the weight at the beginning of incubation and *Wt* is the weight after *t* days of exposure.

Total protein content of the worms was determined by dye-binding method [5] using bovine serum albumin as a standard. Glycogen content was determined using the method described by Carol et al. [9]. Glycogen was separated from soluble sugar by precipitation in the presence of methanol. After centrifugation (15 min, 3,000 rpm), precipitates were used for glycogen quantification with anthron reagent according to the sulfuric acid method of Kemp & Heijningen [22]. Calibration was performed using standard of glucose ranging 0 to 200 µg which received the same treatment as the samples.


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### *Extraction and quantification of pyridine and formaldehyde*

Water, in which *L. minor* and *T. tubifex* were incubated, was analyzed directly for pyridine with High Performance Liquid Chromatography (HPLC; Metrohm, Micro Devices, Switzerland) without pre-concentration. Whereas, for worms, 2 gm of sample from each microcosms, was homogenized in 10 mL of hexane using a glass homogenizer immersed in ice. The homogenate was mechanically shaken for 120 min, filtered and then washed twice with 5 mL of hexane. The combined extract, were then centrifuged at 10,000 rpm for 5 min at $4^0$C and quantitatively transferred to a volumetric flask. The final volume was recorded and the hexane extract stored at $4^0$C. The total extract was evaporated to dryness under a nitrogen stream, the residue was dissolved in 1 mL of acetonitrile and injected into the HPLC [29]. Formaldehyde content in worms, sediment and water was analyzed by spectrophotometrically by formation of diacetyldihydrolutidine (DDL) [32].

## Results

### *Duckweed toxicity test*

Initial fronds number was 100 for each microcosms treated with different concentrations of formaldehyde and pyridine (0-0.9 $mgL^{-1}$). In formaldehyde treated microcosms, concentrations >0.3 $mgL^{-1}$ were found highly growth inhibitor in terms of total chlorophyll, total protein and biomass (*Table 2.*). Even at very low concentration (0.1 $mgL^{-1}$) chlorosis started after 7 days and 20% chlorosis was recorded at 9 days. Whereas, at 0.3 $mgL^{-1}$ formaldehyde, 50% chlorosis was recorded after 12-days exposure. After 18 days, even 0.2 $mgL^{-1}$ formaldehyde showed 100% necrosis. Interestingly, it was observed that at lower concentrations, pyridine (0.1-0.2 $mgL^{-1}$) was growth supportive to *L. minor* in terms of total chlorophyll, total protein and biomass (*Table 2.*).

***Table 2.*** *Effect of pyridine and formaldehyde on total chlorophyll, protein and biomass content of L. minor after 21 days exposures, expressed as percent of control*

| Conc. | **Total chlorophyll** | | **Protein** | | **Biomass** | |
|---|---|---|---|---|---|---|
| ($mgL^{-1}$) | Formaldehyde | Pyridine | Formaldehyde | Pyridine | Formaldehyde | Pyridine |
| 0.0 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| 0.1 | 35.50 [a] ±0.25 | 113.13 [a] ±1.25 | 65.45 [a] ±0.75 | 120.75 [a] ±0.36 | 75.35 [a] ±0.85 | 100.21 [a] ±0.12 |
| 0.2 | 25.75 [a] ±0.15 | 108.60 [a] ±0.98 | 35.25 [a] ±0.36 | 125.07 [a] ±0.45 | 45.15 [a] ±0.25 | 140.28 [a] ±0.68 |
| 0.3 | 5.05 [a] ±0.10 | 90.16 [a] ±1.05 | 5.25 [a] ±0.05 | 115.45 [a] ±0.55 | 10.45 [a] ±0.10 | 105.10 [a] ±0.10 |
| 0.4 | 0.00 | 55.13 [a] ±0.63 | 2.15 [a] ±0.03 | 75.97 [a] ±1.25 | 5.25 [a] ±0.04 | 88.65 [a] ±0.65 |
| 0.5 | 0.00 | 45.51 [a] ±0.45 | 0.00 | 65.25 [a] ±0.88 | 2.35 [a] ±0.05 | 75.25 [a] ±0.75 |
| 0.6 | 0.00 | 30.23 [a] ±0.35 | 0.00 | 45.88 [a] ±0.65 | 0.00 | 48.35 [a] ±0.55 |
| 0.7 | 0.00 | 5.42 [a] ±0.02 | 0.00 | 12.36 [a] ±0.54 | 0.00 | 5.65 [a] ±0.07 |
| 0.8 | 0.00 | 0.00 | 0.00 | 9.25 [a] ±0.08 | 0.00 | 2.88 [a] ±0.03 |
| 0.9 | 0.00 | 0.00 | 0.00 | 5.45 [a] ±0.06 | 0.00 | 2.45 [d] ±0.04 |

Data presented are means (n=3) ± standard deviation; [a] highly significant; ANOVA $p<0.001$, [d] non significant; ANOVA $p>0.05$.

During 6 days of pyridine exposure, no chlorosis as well as necrosis was observed at any concentration. After 9 days of pyridine exposure, chlorosis and necrosis were


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observed at 0.4 $mgL^{-1}$ and 0.5 $mgL^{-1}$, respectively. At the end of experiment (21 days), higher concentrations of pyridine (>0.4 $mgL^{-1}$) showed higher chlorosis (80-100%) as well as necrosis (70-100%) as compared to control. According to the data (*Table 2*.) the 21 day $IC_{50}$ values of pyridine and formaldehyde for total chlorophyll, total protein and biomass are presented in *Table 3*.

*L. minor* exposed to pyridine showed an $IC_{50}$ value 0.45±0.04 for total chlorophyll content, which was 5.8 times of formaldehyde ($IC_{50}$ 0.08±0.002). In terms of protein content, *L. minor* showed an $IC_{50}$ value of 0.58±0.03 for pyridine, which is 3.9 times more than formaldehyde ($IC_{50}$ 0.15±0.02). While, for biomass $IC_{50}$ value for pyridine was found 3.3 times more than formaldehyde (*Table 3*.).

***Table 3.*** *$IC_{50}$ values ($mgL^{-1}$) of pyridine and formaldehyde to L. minor after 21 days*

| Compounds | $IC_{50}$ values for *L. minor* | | |
|---|---|---|---|
| | **Chlorophyll** | **Protein** | **Biomass** |
| Pyridine | 0.45±0.04 | 0.58±0.03 | 0.59±0.02 |
| Formaldehyde | 0.08±0.002 | 0.15±0.02 | 0.18±0.02 |

Data presented are means (n=3) ±standard deviation

## *Tubificid toxicity test*

### *Mortality*

For the control group no mortality was observed. In presence of pyridine and formaldehyde, some mortality was recorded and it increased with the pyridine and formaldehyde concentrations. In formaldehyde treated microcosms, 50% mortality ($LC_{50}$) was recorded at >0.8 $mgL^{-1}$ concentration with in 7 days. Whereas, in pyridine treated microcosms LC values ($LC_{10}$, $LC_{25}$ and $LC_{50}$) were recorded higher than formaldehyde (*Table 4*.)

***Table 4.*** *Lethal concentrations ($mgL^{-1}$) of pyridine and formaldehyde to T. tubifex after 7, 14 and 21 days of exposure.*

| **Compounds** | | **7-days** | **14-days** | **21-days** |
|---|---|---|---|---|
| Pyridine | $LC_{10}$ | 2.66±0.2 | 1.25±0.1 | 0.85±0.08 |
| | $LC_{25}$ | 6.42±0.4 | 3.13±0.3 | 2.14±0.2 |
| | $LC_{50}$ | 12.84±0.2 | 6.25±0.4 | 4.27±0.3 |
| Formaldehyde | $LC_{10}$ | 0.15±0.01 | 0.1±0.01 | 0.08±0.004 |
| | $LC_{25}$ | 0.36±0.02 | 0.24±0.04 | 0.2±0.03 |
| | $LC_{50}$ | 0.73±0.08 | 0.48±0.04 | 0.39±0.05 |

Data presented are means (n=3) ±standard deviation

### *Growth rate*

The growth rate of untreated (control) worms was positive and although it was not significant, it increases from 102 to 155% from 7 day to the 21 day measurement. In contrast, pyridine and formaldehyde treated worms lost weight, as showed in *Fig. 1 & 2*. This decrease of weight was time and concentration dependent. At 0.9 $mgL^{-1}$ concentration of pyridine, the growth rate was reduced to 65±4%, 40±2% and 42±2% after 7, 14 and 21 days of exposure, respectively. While, at 0.9 $mgL^{-1}$ concentration of


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formaldehyde, the reduction was 80±5.4, 60±5 and 62±4% in 7, 14 and 21 days of exposure, respectively (*Fig. 1 & 2.*).

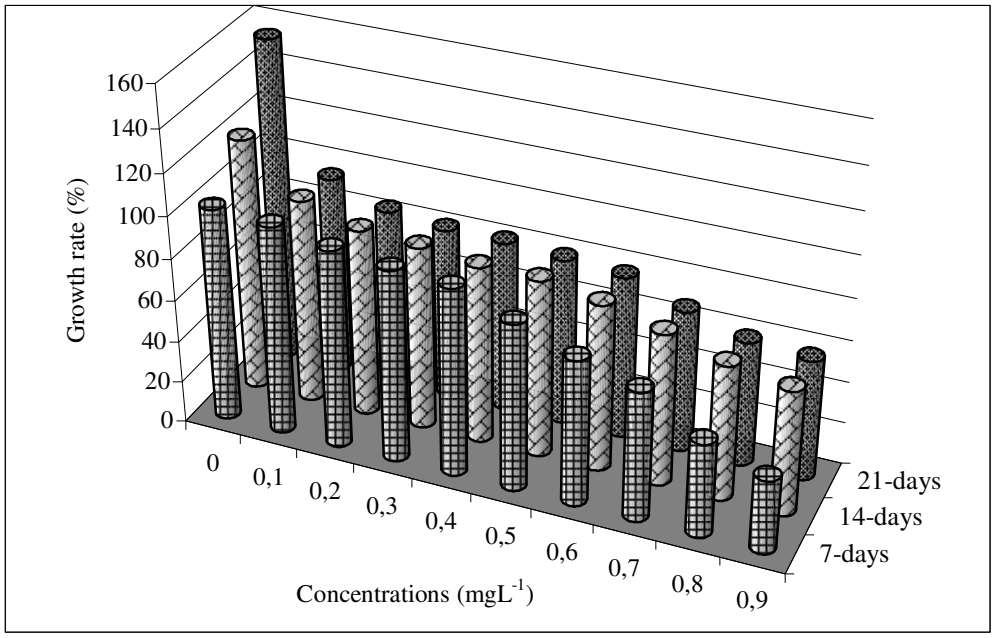


***Figure 1.*** *Effect of various pyridine concentrations on the relative growth rate of T. tubifex after 7, 14 and 21 days of exposure*

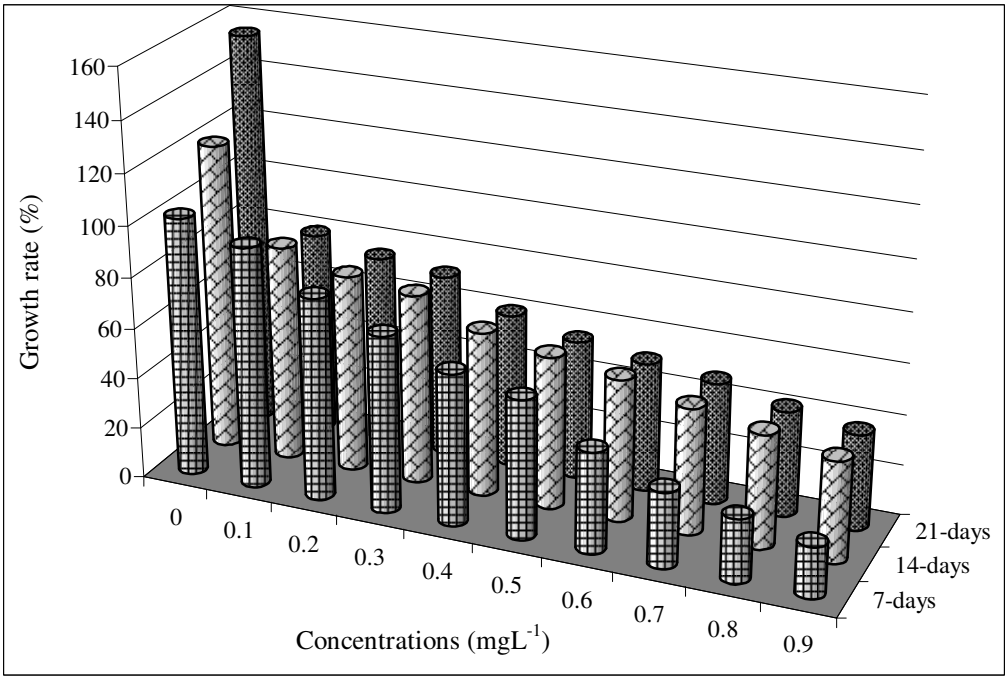


***Figure 2.*** *Effect of various formaldehyde concentrations on the relative growth rate of T. tubifex after 7, 14 and 21 days of exposure*


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*Total protein and glycogen content*

In control *T. tubifex*, the concentration of soluble protein was increased significantly throughout the experiment (1500-2325±24 µg-g $FW^{-1}$). In exposed worms, the decrease of total protein was time and concentration dependent. At higher concentration, pyridine and formaldehyde both significantly reduced ($p < 0.01$) reduced total protein content of worms (*Fig. 3 & 4.*).

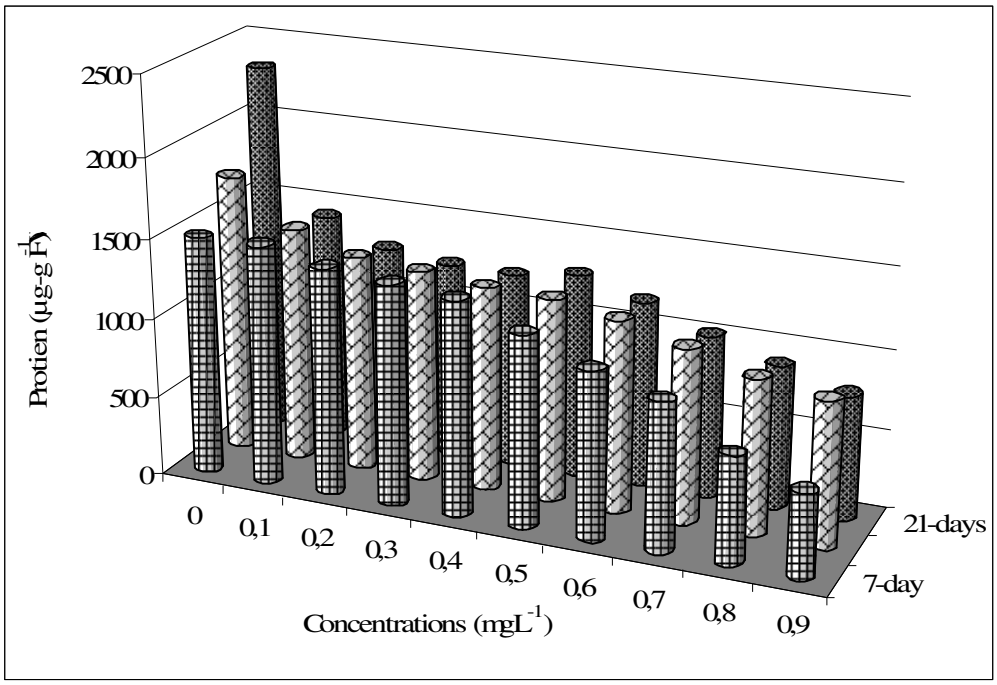


***Figure 3.*** *Effect of various pyridine concentrations on total soluble protein concentration of T. tubifex after 7, 14 and 21 days of exposure*

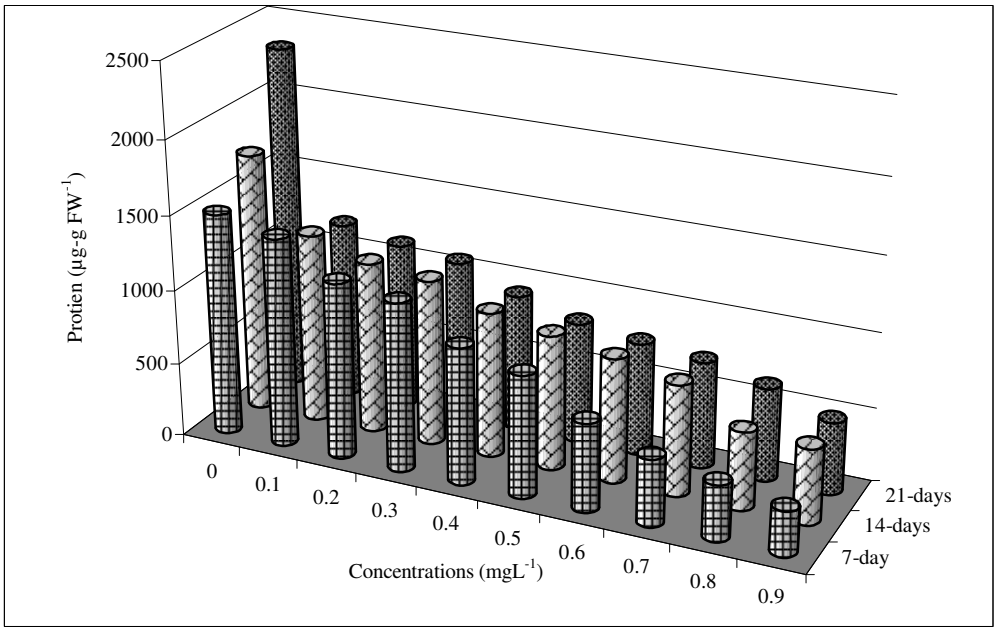


***Figure 4.*** *Effect of various formaldehyde concentrations on total soluble proteinconcentration of T. tubifex after 7, 14 and 21 days of exposure*


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Similarly to protein, the glycogen level in the treated worms was significantly lower than those in control worms, which was approximately 10.47±0.4 to 19.76±1µg-g $FW^{-1}$. This decrease was further concentration and time dependent and reached 34.95±2.5, 59.12±3.2, and 57.97±2.5% after 7, 14 and 21 days in 0.9 $mgL^{-1}$ pyridine treated worms. While, in formaldehyde treated worms, this decrease was reached 19.96±2, 36.63±2.4 and 37.06±2.6% at 0.9 $mgL^{-1}$ concentration (*Fig. 5 & 6*.).

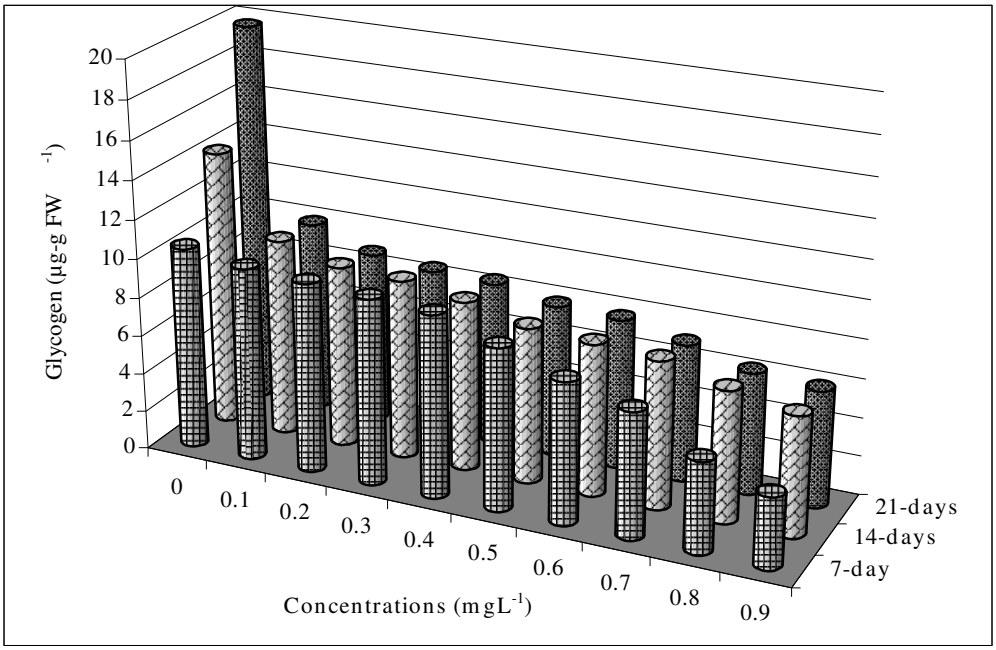


***Figure 5.*** *Effect of various pyridine concentrations on glycogen concentration of T. tubifex after 7, 14 and 21 days of exposure*

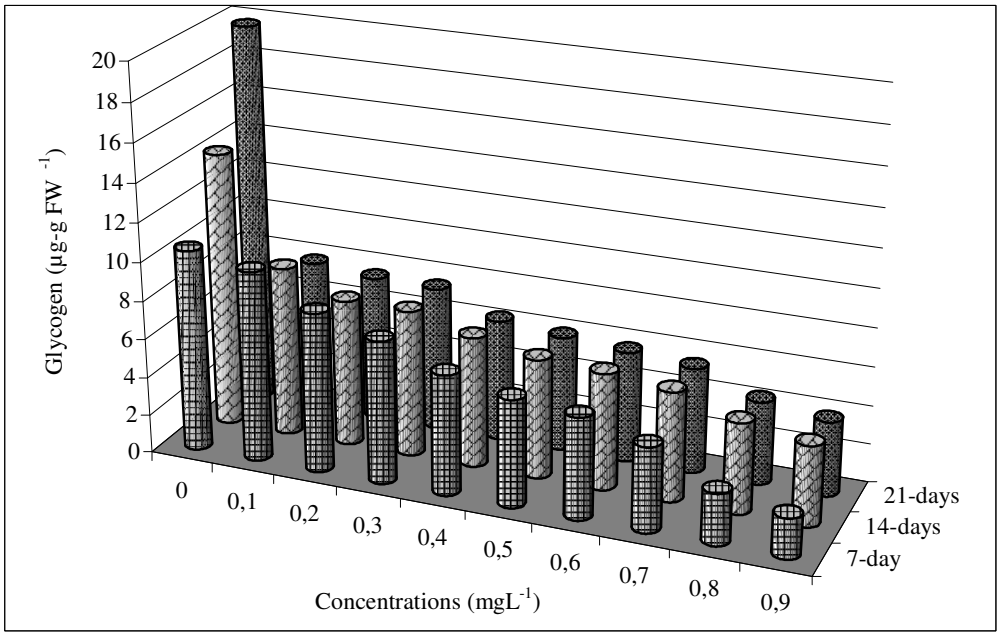


***Figure 6.*** *Effect of various formaldehyde concentrations on glycogen concentration of T. tubifex after 7, 14 and 21 days of exposure*


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*Pyridine and formaldehyde residues*

After 7, 14 and 21 days, residues of pyridine and formaldehyde were detected in all exposed worms and exposure water (*Table 4 & 5.*). In the water, the concentration of both pyridine and formaldehyde was highest after 7 days and decreased thereafter. The relative decrease was more rapid with low initial concentrations of pyridine and formaldehyde. Indeed, after 7 days 80, 90 and 96.66% and after 21 days 50, 50 and 85% of the pyridine was left for the 0.1, 0.5 and 0.9 $mgL^{-1}$ initial concentrations, respectively. While, after 7 days 60, 84 and 91.11% and after 21 days 30, 26, 77.77% of the formaldehyde was left for the 0.1, 0.5 and 0.9 $mgL^{-1}$ initial concentrations, respectively (*Table 4.*).

***Table 5.*** *Residue of pyridine and formaldehyde in exposure water of microcosms ($mgL^{-1}$) in which T. tubifex were incubated during 7, 14 and 21 days*

| **Initial concentration ($mgL^{-1}$)** | **Duration** | | |
|---|---|---|---|
| | **7-days** | **14-days** | **21-days** |
| Control | ND* | ND | ND |
| Pyridine | | | |
| 0.1 | $0.08\pm0.02^{a}$ | $0.06\pm0.01^{b}$ | $0.05\pm0.02^{c}$ |
| 0.5 | $0.45\pm0.04^{d}$ | $0.35\pm0.02^{e}$ | $0.25\pm0.02^{f}$ |
| 0.9 | $0.87\pm0.15^{g}$ | $0.82\pm0.15^{g}$ | $0.77\pm0.20^{h}$ |
| Formaldehyde | | | |
| 0.1 | $0.06\pm0.02^{a}$ | $0.04\pm0.01^{b}$ | $0.03\pm0.01^{c}$ |
| 0.5 | $0.42\pm0.03^{d}$ | $0.31\pm0.05^{e}$ | $0.13\pm0.04^{f}$ |
| 0.9 | $0.82\pm0.25^{g}$ | $0.76\pm0.20^{h}$ | $0.70\pm0.25^{h}$ |

Data presented are means (n=3) ±standard deviation; *ND- not detected; Figures followed by identical superscript letter are not significantly different

***Table 6.*** *Residue of pyridine and formaldehyde in T. tubifex ($\mu g\text{-}g^{-1}$ FW) after 7, 14 and 21 days*

| **Initial concentration ($mgL^{-1}$)** | **Duration** | | |
|---|---|---|---|
| | **7-days** | **14-days** | **21-days** |
| Control | ND* | ND | ND |
| Pyridine | | | |
| 0.1 | $0.06\pm0.01^{a}$ | $0.03\pm0.02^{b}$ | $0.03\pm0.02^{b}$ |
| 0.5 | $0.3\pm0.05^{c}$ | $0.20\pm0.04^{d}$ | $0.16\pm0.02^{e}$ |
| 0.9 | $0.55\pm0.08^{f}$ | $0.60\pm0.05^{g}$ | $0.35\pm0.20^{g}$ |
| Formaldehyde | | | |
| 0.1 | $0.05\pm0.02^{a}$ | $0.03\pm0.01^{b}$ | $0.02\pm0.01^{c}$ |
| 0.5 | $0.28\pm0.03^{d}$ | $0.22\pm0.05^{e}$ | $0.18\pm0.04^{f}$ |
| 0.9 | $0.50\pm0.25^{g}$ | $0.58\pm0.20^{h}$ | $0.28\pm0.25^{i}$ |

All values are mean (n=3) ±standard deviation ; *ND- not detected; Figures followed by identical superscript letter are not significantly different.

In the worms, the concentrations of pyridine reached a maximum of 60 - 61.11% after 7 days for all the initial concentrations used and decreased thereafter, representing 30, 40 and 66.66% after 14 days, 30, 32 and 38.88% after 21 days of exposure of 0.1, 0.5 and 0.9 $mgL^{-1}$ concentrations, respectively. While, the concentration of formaldehyde was found to be 50, 56 and 55.55% after 7 days, 30, 44 and 64.44 after 14


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days and 20, 36 and 31.11% after 21 days at 0.1, 0.5 and 0.9 $mgL^{-1}$ concentrations, respectively (*Table 5*.).

## Discussion

### *Duckweed toxicity test*

*Pyridine toxicity*

Our results suggested that pyridine affects biomass and protein content in the same manner as in the chlorophyll. Due to high water solubility and low octanol-water partition coefficient ($K_{ow}$) (1.04) of pyridine, the cells of *L. minor* have an affinity to pyridine [11]. Since, the pyridine containing herbicides (i.e. diaquat and paraquat) specifically exert phytotoxic action on photosystem II; changes in the chlorophyll content can be a reliable indicator of toxicity [27]. At each lower concentrations, pyridine stimulated production of the chlorophyll, protein and biomass content as well. Mayer and Jensen [28] have also reported triazine, an n-heterocyclic ring containing herbicide, induced increase of the algal chlorophyll and biomass in *Selenastrum capricornatum*. This process may result from homeostasis triggered, as a tolerance mechanism, by the exposure to the herbicides [17]. Responses such as the synthesis of thylakoid components are considered to be a general adaptive response to situations in which the electron transport rate is strongly limited for photosynthesis [3]. An increase in protein content in *L. minor* at lower concentrations of pyridine could also be related with aforesaid detoxification mechanism. For instance, other solvents and herbicides have been shown to be detoxified by various microalgal species and subsequent binding to a protein [24].

*Formaldehyde toxicity*

At lower concentrations, plants inefficiently remove formaldehyde and detoxify or convert it to natural products [19]. The enzyme responsible for formaldehyde detoxification was isolated and characterized as glutathione dependent formaldehyde dehydrogenase forming formic acid. This product is then assimilated into amino acids, sugars and natural products [16]. But at higher concentrations (>0.1%), formaldehyde is very toxic to photosynthetic systems, as well as protein and biomass contents of plant. Due to very low $K_{ow}$ value of formaldehyde (0.35) and high water solubility, the cells of *L. minor* have an affinity for formaldehyde molecules [36]. Once inside the cell formaldehyde inhibits the biological mechanisms i.e. protein synthesis and photosynthesis of plant. But the mode of formaldehyde toxicity in plant is still unknown. In addition, formaldehyde was found approximately three times more toxic than pyridine due to its approximately three times lower $K_{ow}$ value (0.35) than pyridine ($K_{ow}$ value 1.04).

### *Tubifex tubifex toxicity test*

*Pyridine and formaldehyde toxicity*

The values of $LC_{10}$ (2.66±0.2 $mgL^{-1}$ after 7 days, 1.25±0.1 $mgL^{-1}$ after 14 days and 0.85±0.08 $mgL^{-1}$ after 21 days, *Table 4*.) indicated a low toxicity of pyridine towards *T.*


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*tubifex*. While, the of $LC_{10}$ values (0.15±0.01 $mgL^{-1}$ after 7 days, 0.1±0.01 $mgL^{-1}$ after 14 days and 0.08±0.004 $mgL^{-1}$ after 21 days, *Table 4*.) of formaldehyde indicated a moderately high toxicity towards *T. tubifex*.

The growth rate of untreated worms was positive. In contrast, the treated worms lost weights. Reduction in feeding activity as a strategy to avoid the pyridine containing pesticides was proposed to explain the weight reduction in an isopods [37]. In our case, this possibility may be ruled in and it may be proposed that *T. tubifex* growth rate was reduced because of a higher consummation of the reserves.

The reduction of protein content may be ascribed to a catabolism of protein in response to worm energy demand as suggested for an isopod in response to a pyridine containing pesticides [37]. Several authors have shown that the reduction of worm protein content was one of the primary toxic effects of various pesticides, this decrease of protein content appeared to be an early defense reaction to the pesticides stress in animals. To overcome the stress situation, animal require high energy and this energy may have led to protein catabolism. Furthermore, this decrease in protein content might be due to a mechanical lipoprotein formation, which will be used to repair damaged cells, tissues and organs [37]. Similarly to protein, the depletion of glycogen may be due to direct utilization of this compound for energy generation, as a result of pesticide-induced stress [38]. Glycogen is rapidly catabolised, resulting in an important decrease in this energy reserve.

Major fraction of pyridine and formaldehyde was absorbed through the skin (via mucous) due to its high water solubility and minor fraction was entered via food [37] in *T. tubifex*. Once inside the body of worms, pyridine was eliminated through the skin as free-base and as metabolites [6]. The metabolic fate of pyridine in aquatic organisms is still poorly understood. In formaldehyde treated worms, formaldehyde must be first deposited on the outer surface of the mucous blanket for causing toxicity. Mucous is made-up of water [≥95%,] mucous glycoprotein [0.5-1%], free proteins and salts, with other materials in such smaller amount [14]. Before formaldehyde can directly affect the underlining epithelium they must penetrate this superficial layer [41]. Due to their low $K_{ow}$ value formaldehyde binds readily to mucous proteins [18] and polysaccharides [23] thereafter, diffuse through the underlining periciliary fluid and altered the normal functioning of the cell. But, the exact mechanisms and their metabolic fate are still unknown.

***Speciation of formaldehyde and pyridine***

In the environment, decrease in the concentration of a compound in water may be due to adsorption, translocation and degradation. In our experiment, adsorption and translocation can be ruled out due to very low octanol-water partition coefficient ($K_{ow}$) [7, 40] and very low organic content of used sediment (*Table 1*.). Disappearance of the pyridine and formaldehyde in our case is probably due to biodegradation. The slower decrease with high concentrations of pyridine and formaldehyde may be attributed to a possible toxicity to *T. tubifex* and microorganisms that may be present in the medium. Decreasing pyridine and formaldehyde availability in the water may not solely be invoked the concentration decrease in the worms, indeed concentration decrease was not as rapid in the water as in the worms (*Table 4 & 5*). Furthermore, for the highest initial concentration, the pyridine and formaldehyde concentration in the worms increased from day 7 to 14 and it slightly (although not significantly) decreased thereafter. This slight decrease of pyridine and formaldehyde in worms is a defense


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mechanism of *T. tubifex*. Continuous mucous secretion and autotomy of the caudal region are methods by which the animal may protect itself against the increases of internal concentrations of toxic compounds. In addition, 50 to 70% of the administered dose of toxicants was recovered within 24 days of administration [8]. These protection mechanisms appear to be effective, since they led to decontamination of the worms [25].

## Conclusions

This study documents the adverse effects of formaldehyde and pyridine on the chlorophyll, protein and biomass of *L. minor* and mortality, growth, protein and glycogen content of *T. tubifex*. *T. tubifex* can defend themselves from toxic effects of pyridine and formaldehyde by continuous mucous removal and autotomy of the caudal region. Whereas, in absence of well-defined defense system in aquatic plants, formaldehyde and pyridine easily cross the cell membrane and exert a phytotoxic action, especially on the photosynthetic apparatus. So, *L. minor* was found a sensitive test model than *T. tubifex* and can be used as a reliable indicator model for assessment of whole effluent (WET) as well as compounds toxicity. While applicability of the results is limited by the scope and design of the experiments, they shed light on an area in which little research has been done and improve our understanding of the effect of pyridine and formaldehyde in freshwater ecosystems.

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# PROTEIN PROFILES IN WHEAT SEEDLINGS SUBJECTED TO DEHYDRATION STRESS

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**Abstract.** Changes in the soluble protein content, electrophoretic profiles of total and thermostable proteins and polypeptides in endosperms and roots of wheat seedlings germinated under optimal and stress conditions were investigated. Low and high temperature, 0.2 M NaCl, 0.5 M sucrose, 30 μM ABA and 10 mM $H_2O_2$ were applied as stress factors and seeds germinated for 72 hours. The results obtained show that the time course of soluble protein mobilization at early germination depended on the nature of stress factors applied. The response to stress conditions was tissue specific. Low temperature stress elicited the greatest number and most intense total polypeptides in the roots of treated seedlings. High temperature most strongly influenced the thermostable polypeptide profile in endosperms. Content of a 25-kDa polypeptide related to cold tolerance increased in the roots of stressed seedlings. A thermostable polypeptide with a molecular weight of 42 kDa was found in $H_2O_2$ stressed samples that was highly intensive and probably constitutes a stress specific response.
**Keywords:** *ABA, germinating seeds, hydrogen peroxide, polypeptide spectra*

## Introduction

Proteins are compounds of fundamental importance for all functions in the cell [1]. It is well known that alteration of gene expression is always involved in preparing plants for an existence under stress. Protein variation is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions [2],[3]. Under conditions of water deficit (dehydration) numerous processes are modified or impaired [4]. Water stress affects the protein levels of plants but the results of different authors are contradictory. Some authors show decreased protein levels under water stress [5],[6]. Others found an absence of deleterious effects of drought on protein levels [7]. Increasees in protein levels have also been reported [8]. One way of plants to tolerate abiotic stresses to some degree is by biosynthesis of so called stress proteins. Among them are dehydrins that accumulate in plants in response to ABA, low temperature and any other environmental influence that has a dehydration component, such as drought, salinity or extracellular freezing. [9]; [10], [11], [12].

A total of 73 genes encoding proteins were detected by Rabbani *et al.*[13] as stress inducible in rice. Among them 36, 62, 57, and 43 genes were induced by cold, drought, high salinity, and ABA, respectively. Fifteen genes responded to all four treatments.

The kinetics of mobilization of storage protein reserves in germinating seeds under optimal conditions has received considerable attention [14],[15]. The disturbances to this process under stressed conditions are less well understood [16] that means the role of protein mobilization in plant responce to different stresses is still not well understood.


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In order to determine whether seeds have evolved unique mechanisms to deal with environmental stresses we studied the effect of chronic stress on protein and polypeptide profiles in wheat seedlings subjected to different kind of abiotic stresses (extreme temperatures, salinity, osmotic shock). The common feature of all stresses applied is a dehydration component. ABA was used as a dehydration stress confirming standard.

## Materials and methods

### *Plant material and stress treatments*

Wheat seeds (*Triticum aestivum* L, Sadovo 1 cultivar) derived from field grown plants were used throughout the experiments. Sadovo 1 is a Bulgarian high productive cultivar resistant to pathogens, cold and drought. Seeds were germinated in filter paper rolls wetted with tap water, in darkness under optimal (24°C control), low (10°C) or high (38°C) temperature or in the presence of 0.2 M NaCl, 0.5 M sucrose, 30 μM ABA or 10 mM $H_2O_2$, at 24°C. The point at which water (or the treatment solution) was added to "dry seeds" was taken as time zero with respect to the duration of germination. Endosperms and roots of wheat seedlings grown for 72 hours were analyzed.

### *Protein extraction*

Cell-free extracts from seedlings subjected to various treatments were analyzed for soluble protein content, electrophoretic protein and polypeptide spectra on non-denaturing and denaturing gels. Freshly harvested endosperms and roots samples were ground in 0.1 M tris-HCl buffer, pH 7.1. The fresh material / buffer ratio was 1:3 for endosperms, and 1:10 for roots. The homogenate was centrifuged at 12 000 x g for 30 min at 4°C. The supernatant was used for investigations. Protein samples were mixed with equal volumes of 40% sucrose solution (in the case of native PAGE) or sample buffer in (the case of SDS-PAGE) and stored at –20°C.

### *Thermostable protein fraction*

The fraction of thermostable soluble proteins was obtained by the procedure of [17]. The supernatants with soluble proteins were boiled at 100°C for 10 min, stored on ice and then centrifuged at top speed (15 000 x g) in a microcentrifuge for 15 min. The supernatants contained thermostable soluble proteins.

### *Total protein content determination*

Protein content in the crude extracts was determined after TCA precipitation according to the method of Lowry *et al.* [18], using BSA as a standard.

### *Native polyacrylamide gel electrophoresis*

Native PAGE in a 7.5 % cylindrical gels with no reducing or denaturing agents was carried out according to the method of Davis [19]. One hundred μg of protein was loaded per tube and two mA per tube applied during electrophoresis. The gels were stained for protein with Coomassie brilliant blue R-250. The stained protein profiles were scanned densitometrically (ERI-10, Germany) at 600nm. Quantitative differences


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between protein spectra were evaluated by the intensity of the staining of bands and qualitative differences were estimated by the number and Rm values.

### *Rm*

(relative mobility) is the ratio between distance in centimeters from the start of the gel to the place of the protein band on the gel and the distance from the start of the gel to the front (marker dye bromphenol blue).

### *SDS-PAGE*

was conducted on 12.5 % acrylamide gels according to the description of Laemmli [20]. An electrophoresis calibration kit of MBI Fermentas was used to determine the molecular weight of proteins. Protein bands were detected by the silver staining outlined by Nesterenko *et al.* [21].

The data presented in this paper were obtained in three independent experiments.

## Results

The results of total protein content in endosperms could be ordered from greatest to lowest as follows: low temperature >sucrose >NaCl >$H_2O_2$ >control >ABA >high temperature (*Fig. 1*).

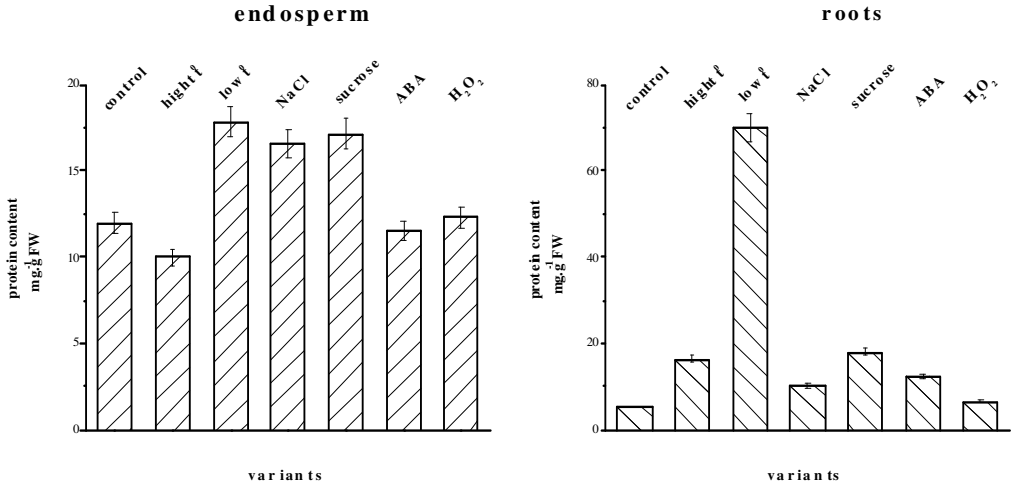


***Figure 1.*** *Protein content of endosperms and roots of 72 hour old wheat seedlings germinated under normal and stressed conditions*

For root samples the order was: low temperature >sucrose >high temperature >ABA >NaCl >$H_2O_2$ >control.

A total of 15 bands ranging in Rm values from 0.05 to 0.66 were observed in the spectrum of total soluble proteins in endosperms (*Fig. 2-A*).


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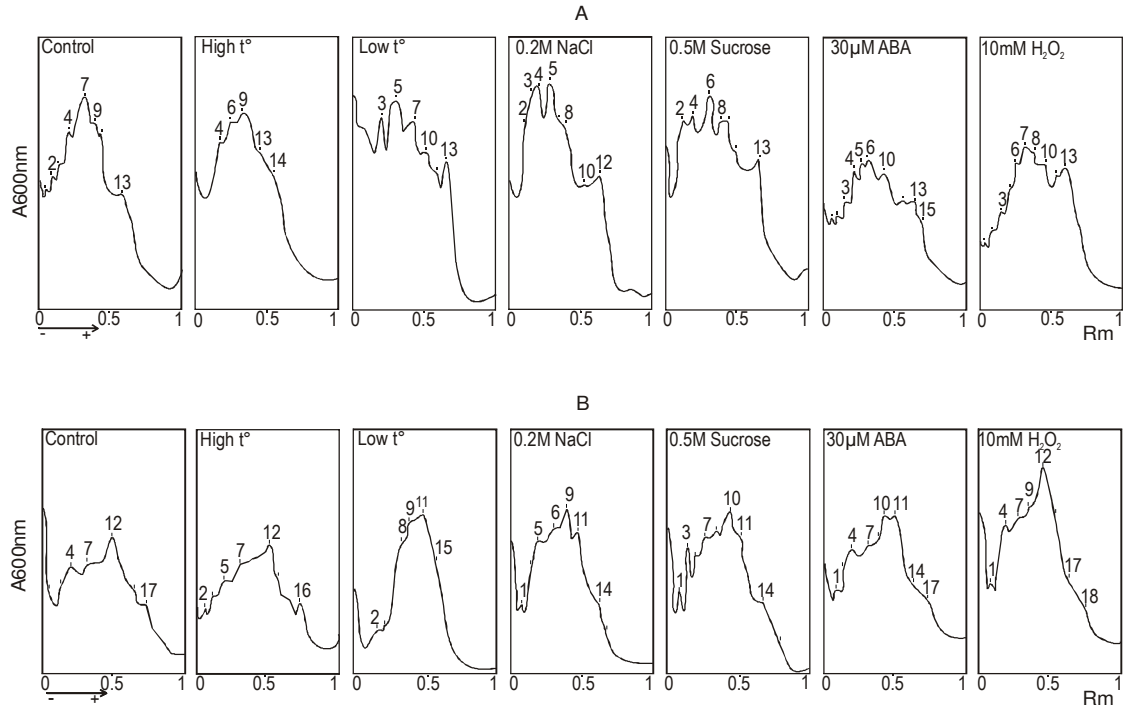


***Figure 2.*** *Electrophoresis of total soluble protein fractions on 7.5% polyacrylamide cylindrical gels after the method of Davis (1964). A – endosperms; B – roots*

Individual treatments contained between 7 (low temperature) and 11 (ABA) different bands. The bulk of the protein across all treatments was located among moderate migrating bands (N 4-10, Rm 0.19-0.44). Eighteen protein bands were distinguished in root samples (*Fig. 2-B*). In contrast to the endosperm samples, in the roots quantitative as well qualitative differences in the total soluble protein spectra of seeds germinated under optimal and stressed conditions were also revealed. The lowest number of bands (6) was observed in the roots of seedlings germinated under low temperature. In the endosperm bands with lower electrophoretic mobility (N 3-9, Rm 0.14-0.39) prevailed, while in the roots moderate migrating bands dominated (N 9-12, Rm 0.39-0.52).

Electrophoretic spectra of thermostable proteins from endosperms of seedlings germinated under stress conditions varied in the quantity and quality of stained bands (*Fig. 3*).

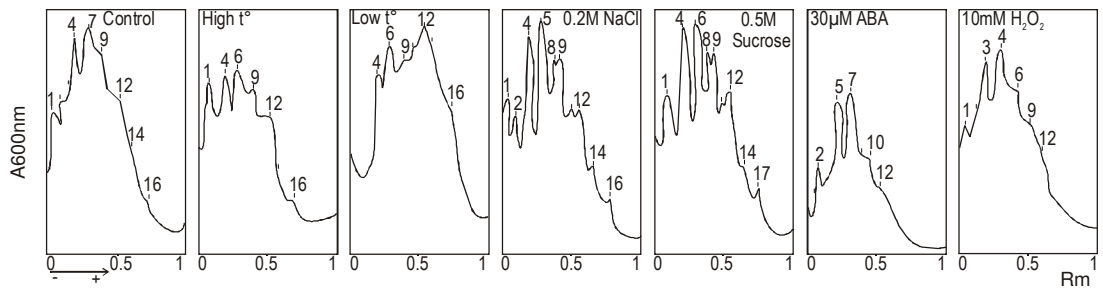


***Figure 3.*** *Electrophoresis of thermostable soluble protein fractions on 7.5% polyacrylamide cylindrical gels*


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Most of the high intensive total protein bands in the endosperms were thermostable. In the endosperms of NaCl and sucrose germinated seedlings numerous sets of intensive bands were detected. In the roots of 72 hours old seedlings thermostable proteins were detected in trace amounts (data are not shown).

Figure 4 shows the greatest number of polypeptides (15) detected in endosperms germinated at low temperature, while the lowest number (10) was found in the endosperm of sucrose-germinated seedlings. The most significant difference however, was the induction of a 65 kDa polypeptide in stressed endosperms that was absent in the control (*Fig. 4*).

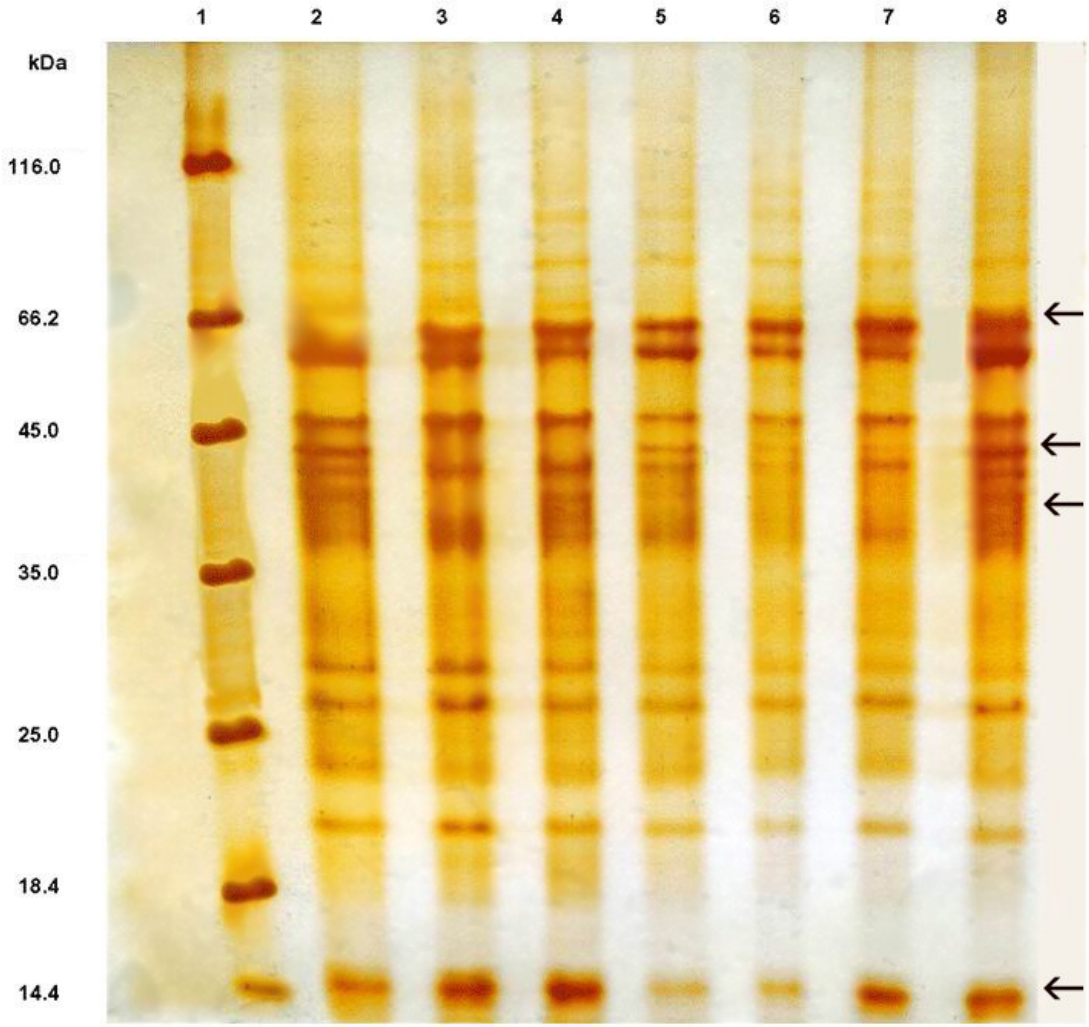


***Figure 4.*** *Polypeptide spectra (SDS-PAGE) of total soluble proteins in the endosperm of seedlings. Lane 1 –molecular mass markers (indicated on the left in kDa) -β-galactosidase; bovine serum albumin; ovalbumin; lactate dehydrogenase; restriction endonuclease Bsp981; β-lactoglobulin; lysozyme, Lane 2 –control, Lane 3 -high temperature, Lane 4 –low temperature, Lane 5 –0.2 M NaCl, Lane 6 –0.5 M sucrose, Lane 7 –30 μM ABA, Lane 8 –10 mM $H_2O_2$. Twenty μg of total protein was loaded in each lane*


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Polypeptides detected in the endosperms following germination segregated into four groups. The groups, based on molecular weight (MW) were: 65-62 kDa, 46-38 kDa, 29-21 kDa and 14.4 kDa. The high MW grouping was most intensive in the endosperm of $H_2O_2$ germinated seedlings followed in decreasing intensity by the high and low temperature, NaCl and ABA treatments. The most intensive polypeptides for the second grouping (46-38 kDa) were detected in $H_2O_2$, control, high and low temperature treatments, while the lowest intensity was in sucrose-germinated seedlings. The three most intensive polypeptide bands in the third MW grouping were found in the high temperature treated endosperms. Finally, the 14.4-kDa band was most intensive in high and low temperature, ABA and $H_2O_2$ treated endosperms, but was weak in the sucrose and NaCl treatments.

Between six and eight thermostable polypeptides were found in control endosperms and all stress treated endosperms. One very intensive thermostable polypeptide with MW 14.4 kDa was found in the endosperms of seedlings tested (*Fig. 5*).

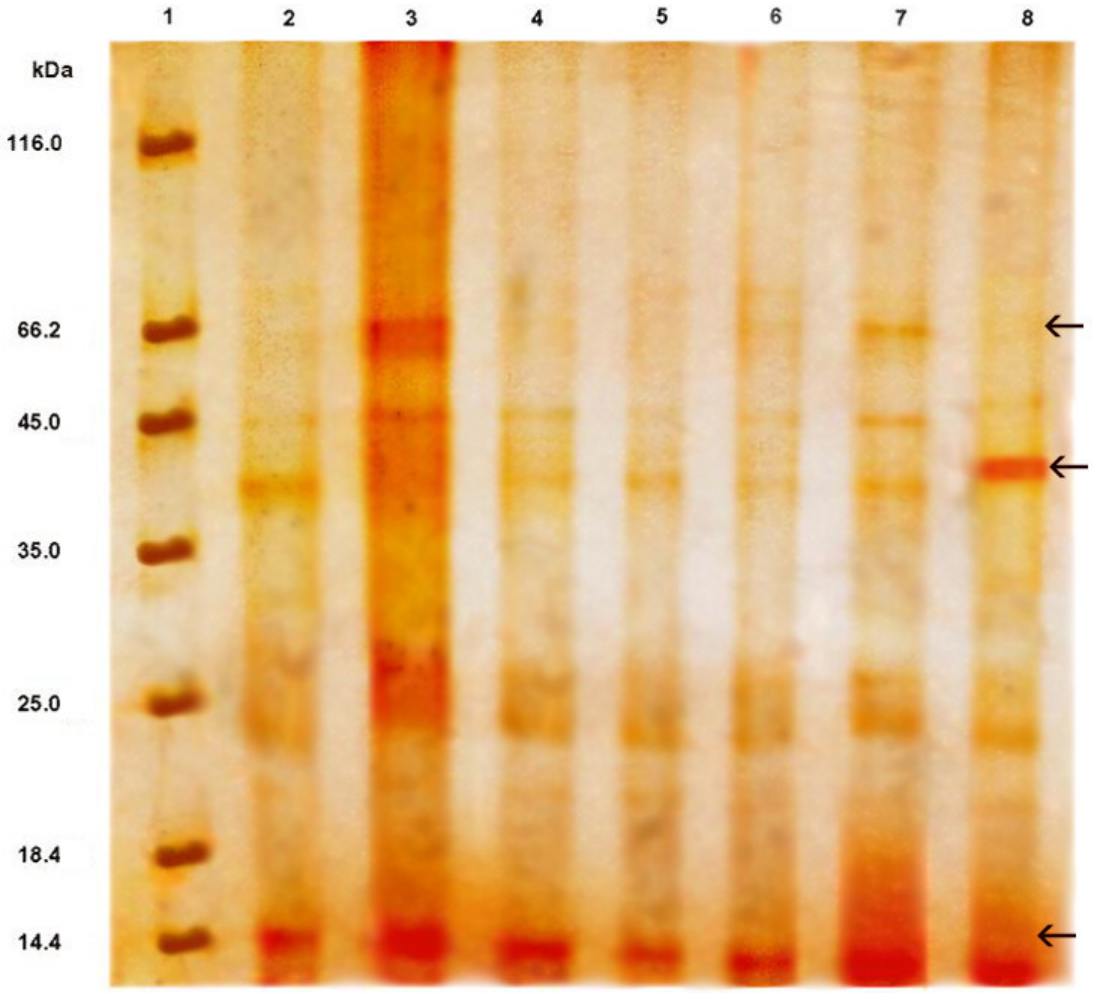


***Figure 5.*** *Polypeptide spectra (SDS-PAGE) of thermostable proteins in endosperm samples. Twenty μg of total protein was loaded in each lane. Lane order as in Figure 4. Molecular mass markers are indicated on the left in kDa*


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The total proportion of thermostable polypeptides was greatest in the endosperm of high temperature treated seedlings. One highly intensive polypeptide band at 42 kDa was found only in the $H_2O_2$ treated endosperms. The high temperature treatment elicited polypeptides with MW of 60 and 66 kDa. The same MW 66 kDa polypeptide was also found as trace amounts in ABA, sucrose and $H_2O_2$ treated endosperms.

In contrast to the endosperms, there was greater variation in the total number of polypeptide bands revealed in root samples.

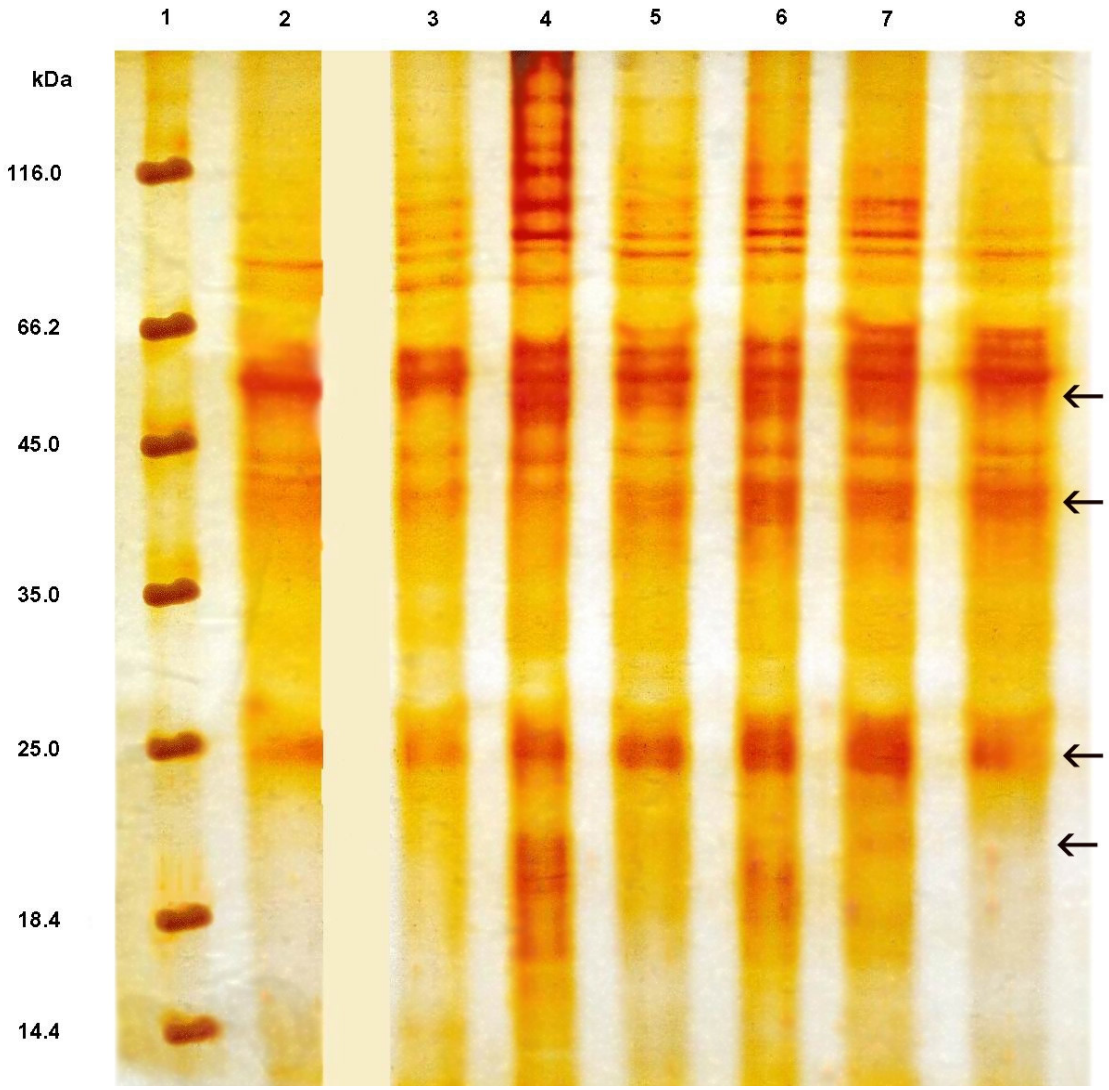


***Figure 6.*** *Polypeptide spectra (SDS-PAGE) of total soluble proteins in the roots of seedlings. Twenty μg of total protein was loaded in each lane. Lane order as in Figure 4. Molecular mass markers are indicated on the left in kDa*

In the roots of control seedlings six total polypeptide bands were revealed (*Fig. 6*), and were most intense at MW of 56 kDa. At high temperature 10 bands were detected and the band intensity pattern was most similar to the control sample. The greatest number (18-19) and the most intensive polypeptide bands were revealed in roots of low temperature germinated seedlings. Bands of particular note included a number above 80 kDa, a group with MW between 60 and 52 kDa, a 25-kDa band and four polypeptides


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with MW between 20 and 17 kDa. Twelve bands were found in roots treated with NaCl during germination. Bands with MW between 60 and 52 kDa and 25 kDa were most intensive. In sucrose treated roots, 16-17 polypeptides stained, with those between 60-52 kDa, 25 kDa and 20-19 kDa being present in the greatest quantity. The same number of bands were present in the ABA treatment, but the spectra was dominated by bands at: 66-52 kDa, 42 kDa, 27 kDa and 25 kDa. Twelve bands were revealed for the $H_2O_2$ treatment, being most intensive at MW of 66-52 kDa, 42 kDa and 25 kDa. It is interesting to note that the highest molecular weight polypeptides (> 80 kDa) were present only in seeds germinated under stressed conditions, being most intense in low temperature treatment. In NaCl, ABA and $H_2O_2$ treated roots a polypeptide with a MW of 66 kDa was detected. Induction of polypeptides between 56-52 kDa occurred in the roots of low temperature, NaCl, sucrose, ABA and $H_2O_2$ treated seeds (*Fig. 6*). Two bands (MW between 45-41 kDa) were present in ABA, sucrose and $H_2O_2$ treated roots, that were more intensive compared to other stress treated seedlings, and were absent in the control. A 25 kDa polypeptide in the roots from seedlings exposed to low temperature, NaCl, ABA and sucrose was significantly more intensive compared to the control and high temperature treated roots. A significant difference between the low temperature stressed roots and other treatments was observed for polypeptides located in a zone at 20-17 kDa. These polypeptides were absent in all other treatments except the sucrose.

## Discussion

There was a higher total protein content in our endosperm samples exposed to low temperature, NaCl, or sucrose stress compared to control samples. We also detected unusually high total protein in root samples exposed to a low temperature treatment of 10°C during germination.

In a previous study we established a retardation of germination rate and reduction of seedling growth as estimated by the reduced FW in all seedlings subjected to low and high temperature, sucrose, ABA, NaCl and $H_2O_2$ treatment. There was a 94 % decrease in growth of roots in low temperature stressed seedlings as compared to the control. The presence of sucrose, high temperature, ABA and NaCl suppressed root growth by 88%, 87%, 78% and 53 %, respectively. In addition, $H_2O_2$ suppressed root growth by 43 %. There were no significant differences between endosperms of seedlings subjected to the stress factors studied [22].

A significant decrease of total protein content during the process of early germination has been established previously in wheat [15], and in triticale [23], [24] seeds germinated under stress- free conditions. In stressed seeds a delay in protein reserve mobilization occurs in maize [25] and pea [16]. These authors reported that the degradation of protein reserves during germination was closely associated with prevailing temperature. Of particular note was that no changes in polypeptide composition occurred in pea embryos imbibing at high temperature (38°C and 40°C) [16]. A suggestion thus arose that a decrease in the activity of proteolytic enzymes may be a contributing factor to the poor germination observed under high temperature stress. Our results for total protein contrast with these observations in pea in that we noted a significant depression of protein degradation at low, not high, temperature in the roots of young seedlings and to a much lesser degree in endosperm tissue. Protein degradation in early germination process was well balanced with the synthesis of new


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polypeptides, which began during the initial hours of embryo imbibition [26]. We propose that the high protein content detected in roots at low temperature was due to the suppression of protein mobilization rather then to protein synthesis based on the fact that seedling growth under low temperature conditions was strongly reduced. This conclusion is based on two premises. Firstly that cold stress inhibits DNA replication, gene transcription and translation [27]. Secondly, Minamikawa *et al*. [28] demonstrated that the degradation of most of globulin proteins at the embryonic axis is likely to be dependent on *de novo* synthesis of proteolytic enzymes.

We observed high molecular weight polypeptides (> 80 kDa) only in seeds germinated under stress conditions. In the control seeds they were absent. Chumikina *et al*., [24] revealed that under optimal conditions (control seedlings) at this stage of germination the same proteins had already been degraded.

We observed high qualitative and quantitative specificity between polypeptide spectra and stress treatments. Stress parameters we have previously investigated (growth and antioxidant enzymes, Bakalova *et al.* [22]) revealed high similarity between control and $H_2O_2$ treatment and also between low temperature and sucrose treatment. It is noteworthy that the lowest number of low intensive polypeptides was observed in sucrose. It could be noted that the polypeptides from first and second group were most intensive in the endosperm of $H_2O_2$ treatment compared with other treatments and also to other polypeptides.

We found polypeptides in the endosperms and roots of wheat germinated under stress conditions ranging from 150 to 14.4 kDa. Four grouping of polypeptides between 14.4 and 66.2 kDa detected in endosperms and five groupings between 18 and 150 kDa were noted in root samples. 72 hours after the onset of imbibition under normal conditions the mobilization of embryo reserves should be almost complete and mobilization of endosperm reserves just begining [14], [15], [24]. In stressed seedlings there were different amounts of high molecular weight polypeptides (*Fig. 6*) that could be due to delayed mobilization and this was especially well pronounced in the case of low temperature treatment. There is much data in the literature to indicate that embryos become self-supporting relatively early on in the germination process [28], [24] through the manufacture and utilization of their own protein reserves. Qualitative and quantitative changes in polypeptide composition in wheat embryos begin around 24 hours after the onset of germination when root growth has begun.[29]. Visible changes in the endosperm begin in triticale after about 96 hours of germination [24]. Our results show that the biochemical composition of roots and endosperms were shown to exhibit certain differences. The roots contained polypeptides of over 66 kDa, while the endosperm contained a low molecular polypeptide with MW 14.4 kDa that was entirely absent in the root. The most significant difference however, was the induction of a 65 kDa polypeptide in stressed endosperms that was absent in the control

Our spectra of total polypeptides highlighted differences between treatments in both roots and endosperms. We found that the time course of protein mobilization is different depending on the particular stress factor applied. In particular we noted tissue specific responses to extreme temperatures. Low temperature stress elicited the greatest number and concentration of polypeptides in roots, and which coincided with a high total protein content observed in the roots. Polypeptide profiles in endosperms were most strongly affected by high temperature. This variance in response to high and low temperature according to tissue type is similar to the variable responses reported by Fowler and Tomashov [30] and Sung *et al.* [31]. The content of thermostable


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polypeptides in the endosperm of high temperature treated seedlings was greater than those germinated at low temperature. Our observations of an absence of thermostable proteins in the roots of 72 hour old seedlings concurs with the results found in other species such as soybean, where thermostable proteins in seedlings decreased and disappeared after 18 hours of germination [32].

One possible explanation for delayed protein mobilization in response to ABA stress is suggested by the data on cereals presented by Fincher [33]. The data suggest that ABA-induced proteins include inhibitors of key enzymes necessary for the germination process. ABA inducible genes could mediate a transient arrest in endosperm protein mobilization in response to unfavorable environmental conditions such as water, temperature and salt stress. We observed a total number of 6-8 thermostable polypeptides in our endosperm samples and a polypeptide with MW 25 kDa was very intensive in stressed endosperms, especially in the high temperature treatment. Marian *et al*. [12] observed in Rhododendron species the same number of dehydrins (thermostable proteins) with MW between 25 and 73 kDa. Our data are consistent with their proposal for a key role of dehydrin with MW 25 kDa in protecting Rhododendron leaves from freezing injury. Data on the correlation between 25 kDa dehydrin accumulation and the level of freezing tolerance were also presented for other species like blueberry [34] and wheat [35]. Our data show that this dehydrin may play protective role in other stress factors like high salinity, high osmoticum, and high temperature.

The conclusions of this study are that low temperature stress elicited the greatest number and the most intensive total polypeptide spectra in the roots of treated seedlings. High temperature most strongly influenced the profile of thermostable polypeptides in the endosperm of seedlings. The time course of protein mobilization depended on the stress factors applied. The amount of a 25 kDa polypeptide (dehydrin) increased in the roots of stressed seedlings. A thermostable polypeptide with a molecular weight of 42 kDa was found in $H_2O_2$ stressed samples that was highly intensive and probably constitutes a stress specific response like protector against oxidative stress.

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# EFFECT OF MOISTURE CONTENT VARIATION OVER KINETIC REACTION RATE DURING VERMICOMPOSTING PROCESS

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**Abstract.** Even though vermicomposting is an age old process, due to rapid changes in technology in present scenario it is essential to change such process with application of advanced technique available. The same attempt is made by authors during series of their experiments of bioconversion over solid waste containing substrates cellulose into useful biofertilizer. In the present study the substrate sugarcane bagasse taken as carbon source is bio-converted with species of earthworm *Eudrilus eugeniae*. The role of moisture content in the process is studied by varying moisture content from 45 ± 5%, 55 ± 5%, 65 ± 5%, 75 ± 5% to 85 ± 5%. It is observed that the moisture content 75 ± 5% is the optimal at which the vermicomposting is fastest. Other relative parameters observed varied with maximum changes in range of 75 ± 5% of moisture content. The maximum kinetic reaction rate is recorded at the same level of moisture content.
**Keywords:** *Cellulose, bagasse, Eudrilus eugeniae, Absolute BOD, C/N*

## Introduction

Worms not only reduce organic matter but also stabilize organic matter and mineralize different elements present in substrate. Mineral rich organic manure in form of compost or vermicompost can be a good replacement to chemical fertilizer. Both ways of conversion of organic waste into useful manure is not difficult but need some awareness and technical knowledge to hold control over the process of bioconversion. Besides recycling and agricultural value, tissues of worms have tremendous medicinal value in China, Japan, Malaysia, and Myanmar, including India [34, 40 & 50]. Earthworms are mainly classified as epigic i.e. shallow burrowing [27 & 49], aneciques i.e. deep burrowing [5] and endoges i.e. surface dwellers [1, 4, 10, 16, 27, 29 & 39].


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## Review of literature

In many countries scientist and engineers have developed the technique to stabilize and manage generated solidwaste by communities in cities. Few processes include the Beccari Process of Italy, Boardas Process, Earp Thamos Process, Freser Process, Hardi Process and others of Europe, the Deno Process developed in Denmark and the Vam Process used in Holland etc. [8]. The first modern development of composting technique can be traced to the work of Howard in the early part of 20th century. Work on these lines was initiated as early as 1920 by the collaborative efforts of Jackson and Wad and others. But are not much fruit full, because they fail to patch nutritive demand of plant life. Indirectly it proved a device to reduce pollution potential up to certain limit. On the other hand vermicomposting is a process which reduces the pollution potential together with fulfilling the nutritive demand of plant for better yield. Presence of worm-cast in agriculture field not only enhances the fertility of land but also increase the water holding capacity of agriculture land. Full knowledge of earthworm species and its behavior corresponding to environmental parameters must be well known before the application to a specific substrate. The gut of earthworm itself works as bioreactor, where the fragmentation and grinding of substrate, bioconversion of substrate and mineralization of different elements present in substrate takes place. This is termed as vermicompost with rich nutritive value. The excreted material from [5, 12, 28, 34, 44 & 45] earthworm is highly soluble in water and is easily accepted by plant for their healthy growth. In the whole process presence of moisture content play important role in the fragmentation, the bioconversion and the mineralization. Mobility of worms also depends on moisture content [34]. The marking of optimal moisture content for earthworm is very important to get faster, economical and eco-friendly results. Many earthworm species are suitable for organic solid waste disposal. In the present study exotic species of earthworm *Eudrilus eugeniae* community known as "African Night Crawler" was used. This species is reported to be fast growing; ferocious eater of organic food and does not need much soil as substratum. The optimal temperature tolerance ranges from 8 °C to 30 °C for best results [15]. After the cocoons of earthworm were deposited, hatching of worms at 25 °C occurs for three weeks and then due to availability of abundant food and space, they grow to a maximum weight of 2.5 g in 8 to 10 weeks thereafter most of them decline in size and slowly attain mortality at the age of 12 weeks. Only 35% to 45% of the individual survive up to 17th week [5, 7, 14, 16, 17, 27, 31 & 34]. Various studies show that endoges species i.e. surface dweller earthworm are most competent than others. The earthworms have 75% to 90% water of their body weight [21]. This indicates that the whole enzymatic reactions are mainly based on moisture content. Optimal level of moisture content is essentially required for vermicomposting (survival of warms and optimal biological activity)as well as for microbial flora present in process [22]. Under adverse dry conditions of soil the worms release large amount of water from their bodies for their survival, and activity of worms get reduced [17].

## Materials and methods

For experimental study the authors selected six spherical earthen pots (generally used to store drinking water in India) of about 50 cm diameter in middle and open mouth of about 25 cm diameter. With the help of power drill, twelve bores of 8 mm diameter


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were drilled below neck of earthen pot at 20 cm slant depth from the neck. Each bore was made approximately at 30 degree angle. Oval shape pebbles (collected from river bed) 10 mm to 25 mm in size were laid at the bottom of earthen pot to create 5 cm height. Stabilized slurry of cow dung from biogas plant was taken and mixed with agriculture soil in the ratio of. 1:1 and put over the stone bed to fill the voids and to prepare a uniform bed (substratum or plate form). Sugarcane bagasse as substrate and agriculture soil are taken and dried in oven at 550 °C and 650 °C respectively for 24 hours to expel out moisture from both. After 24 hours the both were taken out from oven and mixed in the ratio of 64%:36% by weight. This mixture was weighed in six lots of 14 kg and placed in each earthen pot. A level strip was stuck over each pot and marked as control lot, experimental lot, moisture content level, date of experiment, weight of substrate used, weight of earthworm loading, interval and days of sample collection. Record of progress in each pot was thus maintained. Out of the six pots, No 1 marked as control has moisture content maintained at 75 ± 5 % without earthworm. In the pot No 2 to pot No 6 the moisture content level is maintained at 45 ± 5 %, 55 ± 5 %, 65 ± 5 %, 75 ± 5 % and 85 ± 5 % respectively with a level difference of 10 % approximately in each. This level of moisture content was checked, time to time and whenever it was observed that the moisture content level reduced in any pot the moisture content level was immediately patched by sprinkle of appropriate amount of water to recover moisture lost. True representative sample from each pot was drawn periodically and estimated for selected parameters. The *Eudrilus eugeniae* species of earthworm weighing 150 g was introduced in each experimental pot and cotton cloth net was used to cover open mouth and tied at neck of each pot to protect from fly nuisance. Earthworms were kept for 2 to 3 weeks in same composition of substrate for acclimatization. The estimation of moisture content was done with the help of quick moisture meter, cellulose by Updegraph Method (1966), Organic Carbon by Dichromate Method, Total Nitrogen by K-Jeldahal Method respectively. BOD of solid waste was measured with a slight modification in the methodology. 5 g of substrate sample was taken and crushed up to the colloidal particulate size with the help of mechanical device. 1 g by dry weight of this sample was taken and mixed with 1 liter of distilled water of known BOD in volumetric flask and the BOD at 200C of solution was estimated by dilution technique. Wherever needed mathematical calculation were done to get the result. The BOD obtained is expressed in (%) percentage. Absolute BOD at any instance of experiment was determined with respect to dry weight of substance at that particular stage and finally this absolute BOD was used to determine Kr (Reaction rate) values by plotting graph between Log Lt/Lo against time in days.

## Results

### *Cellulose reduction*

The observed results of experiment are presented in Table 1 to Table 6. The initial cellulose level in control lot was 440.20 mg/g which reduced to 385.89 mg/g which shows a poor decrease in cellulose content of total 12.34 % in 48 days. While in experimental lots the gradual increase in percentage cellulose reduction was observed with increase in moisture content. The maximum reduction in cellulose content was observed at 75 ± 5 % moisture content which was initially 440.80 mg/g reaching a level of 32.8 mg/g on 48th day of experimentation. The decrease of cellulose by 92.56% is maximum among all the moisture content levels, the next lower reduction in 48 days


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was observed in 85 ± 5 % followed by that in 65 ± 5 % moisture content in experimental lot. This is a clear indication that after a level of 75 ± 5 % with increase or decrease in moisture content the cellulose reduction get affected.

### *C/N reduction*

The C/N ratio initially was 58.118 in control lot and at the end of experiment reached to a level of 51.068. It shows a poor reduction in C/N ratio i.e. 12.13% while in Table 2 i.e. for 45± 5 % experimental lot better reduction of C/N ratio i.e. 58.07% was observed. Similarly Table 3, 4, 5 shows gradual increase in C/N ratio reduction i.e. 59.91%, 63.85 %, 66.10% respectively. Table No 6 i.e. 85 ± 5 % moisture content level indicates poor reduction in C/N ratio and reduction of 64.09% nearly equal to the 65 ± 5 % moisture content level. It is clear that C/N ratio reaches below 20 in 75 ± 5 % moisture content of experimental lot only.

### *Dry weight reduction*

Dry weight reduction of substrate bagasse was recorded. Increased moisture content in experimental lot from 45± 5 % to 75± 5 % showed increased reduction in dry weight from 22.9 %, 24.9 %, 25.32 % and 28.57 % respectively. Poor percentage reduction in dry weight was recorded in control (13.69 %). The percentage dry weight reduction shows a declining trend as moisture content level reaches higher range of 85± 5 %. In this percentage reduction of dry weight was nearly 2% lower than percentage reduction in lot No. 5 i.e. of moisture content level 75± 5 %.

### *Absolute BOD reduction*

Comparative higher reduction in Absolute BOD values were recorded in lot No 5 at 75± 5 % moisture content. The maximum percentage reduction in Absolute BOD was observed to be 78.15% in lot No. 5, while 71.63%, 72.81%, 74.53% and 75.73% Absolute BOD reduction were recorded in lot No 2, 3, 4 and 6 respectively. This parameter also shows about 2% variation in each successive lot. A poor reduction in % Absolute BOD was recorded in control lot whose moisture content level was similar to experimental lot No. 5 showing greater reduction.

### *Kinetic reaction rate and weight of earthworms*

Kinetic reaction rate of all experimental lots were compared. It was observed that the maximum reaction rate was recorded in lot No. 5 i.e. 0.013, while in control lot No. 1 it was recorded as 0.0001 which is lowest amongst the lots. The kinetic reaction rate shows the gradual increment as moisture content level increases in experimental lots from 2 to 4 i.e. 0.011, 0.0114, 0.012 respectively and attained a maximum in lot No. 5 i.e. 0.013. It is reduced in lot No. 6 i.e. 0.012 which shows a decline phase in reaction rate at higher moisture content level i.e. 85± 5 %. At the completion of experiment weight of earthworms of each experimental lot were recorded. Recorded weight was higher than in the beginning. The weight of earthworms gets increased by 14 %, 20%, 24.7%, 48% and 34% respectively in lot No. 2 to 6 respectively. This promises that the maximum bioconversion happened in lot No 5 whose moisture content level was 75± 5 %.


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***Table 1.*** *Observation on bioconversion of bagasse substrate by Eudrilus eugeniae earthworm species (Control lot)*

| Time days | Cellulose mg/g | C/N ratio | Dry Weight kg | BOD % | Absolute BOD kg |
|---|---|---|---|---|---|
| 0.00 | 440.20 | 58.12 | 14.00 | 38.20 | 5.35 |
| 4.00 | 438.60 | 57.66 | 13.95 | 38.13 | 5.32 |
| 8.00 | 434.90 | 57.12 | 13.89 | 38.08 | 5.29 |
| 12.00 | 431.80 | 56.68 | 13.78 | 38.01 | 5.24 |
| 16.00 | 427.80 | 56.19 | 13.56 | 37.94 | 5.15 |
| 20.00 | 422.40 | 55.67 | 13.42 | 37.88 | 5.08 |
| 24.00 | 417.80 | 55.13 | 13.29 | 37.76 | 5.02 |
| 28.00 | 412.60 | 54.54 | 13.19 | 37.65 | 4.97 |
| 32.00 | 407.30 | 54.08 | 13.09 | 37.55 | 4.92 |
| 36.00 | 402.00 | 53.66 | 12.99 | 37.43 | 4.86 |
| 40.00 | 397.30 | 53.01 | 12.90 | 37.32 | 4.82 |
| 44.00 | 391.80 | 52.10 | 12.89 | 37.21 | 4.80 |
| 48.00 | 385.90 | 51.07 | 12.80 | 37.06 | 4.74 |

* Moisture Content level kept 75 ± 5% through experiment period. (Control lot)
** Agriculture Soil 36% + Sugarcane Bagasse Substrate 64 % by weight
*** Earthworm weight *Eudrilus eugeniae* initial weight 0 gm at the beginning of experiment
**** Earthworm weight *Eudrilus eugeniae* final weight 0 gm at the end of experiment i.e. 00% increase in weight

***Table 2.*** *Observation on bioconversion of bagasse substrate by Eudrilus eugeniae earthworm species (M C 45 ± 5% experiment lot)*

| Time days | Cellulose mg/g | C/N ratio | Dry Weight kg | BOD % | Absolute BOD kg |
|---|---|---|---|---|---|
| 0.00 | 438.90 | 56.94 | 14.00 | 38.82 | 5.43 |
| 4.00 | 430.30 | 56.14 | 13.97 | 38.71 | 5.41 |
| 8.00 | 420.50 | 53.58 | 13.85 | 37.46 | 5.19 |
| 12.00 | 406.00 | 50.63 | 13.66 | 35.71 | 4.88 |
| 16.00 | 397.20 | 47.22 | 13.26 | 33.39 | 4.43 |
| 20.00 | 386.20 | 43.43 | 13.02 | 30.67 | 3.99 |
| 24.00 | 365.20 | 37.96 | 12.68 | 26.40 | 3.35 |
| 28.00 | 338.80 | 34.27 | 12.21 | 21.35 | 2.61 |
| 32.00 | 297.20 | 31.41 | 11.84 | 18.28 | 2.17 |
| 36.00 | 248.30 | 29.04 | 11.55 | 16.38 | 1.89 |
| 40.00 | 158.30 | 26.69 | 11.16 | 15.53 | 1.73 |
| 44.00 | 109.70 | 24.44 | 10.81 | 14.64 | 1.58 |
| 48.00 | 78.60 | 22.83 | 10.51 | 14.05 | 1.48 |

* Moisture Content level kept 45 ± 5% through experiment period (Experimental lot)
** Agriculture Soil 36% + Sugarcane Bagasse Substrate 64 % by weight
*** Earthworm weight *Eudrilus eugeniae* initial weight 150 gm at the beginning of experiment
**** Earthworm weight *Eudrilus eugeniae* final weight 171 gm at the end of experiment i.e. 14 % increase in weight


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***Table 3.*** *Observation on bioconversion of bagasse substrate by Eudrilus eugeniae earthworm species (M C 55 ± 5% experiment lot)*

| Time days | Cellulose mg/g | C/N ratio | Dry Weight kg | BOD % | Absolute BOD kg |
|---|---|---|---|---|---|
| 0.00 | 438.90 | 56.94 | 14.00 | 38.82 | 5.43 |
| 4.00 | 430.30 | 56.14 | 13.97 | 38.71 | 5.41 |
| 8.00 | 420.50 | 53.58 | 13.85 | 37.46 | 5.19 |
| 12.00 | 406.00 | 50.63 | 13.66 | 35.71 | 4.88 |
| 16.00 | 397.20 | 47.22 | 13.26 | 33.39 | 4.43 |
| 20.00 | 386.20 | 43.43 | 13.02 | 30.67 | 3.99 |
| 24.00 | 365.20 | 37.96 | 12.68 | 26.40 | 3.35 |
| 28.00 | 338.80 | 34.27 | 12.21 | 21.35 | 2.61 |
| 32.00 | 297.20 | 31.41 | 11.84 | 18.28 | 2.17 |
| 36.00 | 248.30 | 29.04 | 11.55 | 16.38 | 1.89 |
| 40.00 | 158.30 | 26.69 | 11.16 | 15.53 | 1.73 |
| 44.00 | 109.70 | 24.44 | 10.81 | 14.64 | 1.58 |
| 48.00 | 78.60 | 22.83 | 10.51 | 14.05 | 1.48 |

* Moisture Content level kept 55 ± 5% through experiment period (Experimental lot)
** Agriculture Soil 36% + Sugarcane Bagasse Substrate 64 % by weight
*** Earthworm weight *Eudrilus eugeniae* initial weight 150 gm at the beginning of experiment
**** Earthworm weight *Eudrilus eugeniae* final weight 180 gm at the end of experiment i.e. 20% increase in weight

***Table 4.*** *Observation on bioconversion of bagasse substrate by Eudrilus eugeniae earthworm species (M C 65 ± 5% experiment lot)*

| Time days | Cellulose mg/g | C/N ratio | Dry Weight kg | BOD % | Absolute BOD kg |
|---|---|---|---|---|---|
| 0.00 | 442.80 | 57.03 | 14.00 | 38.85 | 5.44 |
| 4.00 | 434.00 | 56.21 | 13.97 | 38.73 | 5.41 |
| 8.00 | 420.70 | 53.54 | 13.83 | 37.37 | 5.17 |
| 12.00 | 407.60 | 50.32 | 13.64 | 35.55 | 4.85 |
| 16.00 | 396.30 | 46.65 | 13.19 | 33.02 | 4.36 |
| 20.00 | 385.30 | 42.43 | 12.19 | 29.91 | 3.85 |
| 24.00 | 363.30 | 36.80 | 12.57 | 24.86 | 3.13 |
| 28.00 | 335.20 | 32.12 | 12.17 | 20.09 | 2.44 |
| 32.00 | 291.00 | 29.28 | 11.75 | 17.52 | 2.06 |
| 36.00 | 239.10 | 26.84 | 11.34 | 15.62 | 1.77 |
| 40.00 | 141.70 | 24.45 | 10.97 | 15.15 | 1.66 |
| 44.00 | 88.60 | 22.22 | 10.51 | 13.86 | 1.46 |
| 48.00 | 62.10 | 20.62 | 10.46 | 13.25 | 1.39 |

* Moisture Content level kept 65 ± 5% through experiment period (Experimental lot)
** Agriculture Soil 36% + Sugarcane Bagasse Substrate 64 % by weight
*** Earthworm weight *Eudrilus eugeniae* initial weight 150 gm at the beginning of experiment
**** Earthworm weight *Eudrilus eugeniae* final weight 196 gm at the end of experiment i.e. 23.47 % increase in weight


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***Table 5.*** *Observation on bioconversion of bagasse substrate by Eudrilus eugeniae earthworm species (M C 75 ± 5% experiment lot)*

| Time days | Cellulose mg/g | C/N ratio | Dry Weight kg | BOD % | Absolute BOD kg |
|---|---|---|---|---|---|
| 0.00 | 440.80 | 57.18 | 14.00 | 38.90 | 5.45 |
| 4.00 | 431.60 | 56.25 | 13.97 | 38.76 | 5.41 |
| 8.00 | 418.40 | 53.30 | 13.82 | 37.30 | 5.16 |
| 12.00 | 404.90 | 49.80 | 13.60 | 35.10 | 4.77 |
| 16.00 | 390.60 | 45.10 | 13.14 | 32.30 | 4.32 |
| 20.00 | 374.30 | 40.00 | 12.80 | 28.42 | 4.64 |
| 24.00 | 354.20 | 34.32 | 12.38 | 23.19 | 2.87 |
| 28.00 | 319.00 | 28.50 | 11.91 | 18.03 | 2.14 |
| 32.00 | 256.80 | 25.60 | 11.46 | 15.86 | 1.82 |
| 36.00 | 194.60 | 23.19 | 10.96 | 14.02 | 1.54 |
| 40.00 | 124.40 | 21.39 | 10.50 | 13.13 | 1.38 |
| 44.00 | 52.60 | 20.35 | 10.03 | 12.15 | 1.22 |
| 48.00 | 32.80 | 19.38 | 10.00 | 11.90 | 1.19 |

* Moisture Content level kept 75 ± 5% through experiment period (Experimental lot)
** Agriculture Soil 36% + Sugarcane Bagasse Substrate 64 % by weight
*** Earthworm weight *Eudrilus eugeniae* initial weight 150 gm at the beginning of experiment
**** Earthworm weight *Eudrilus eugeniae* final weight 222 gm at the end of experiment i.e. 48% increase in weight

***Table 6.*** *Observation on bioconversion of bagasse substrate by Eudrilus eugeniae earthworm species (M C 85 ± 5% experiment lot)*

| Time days | Cellulose mg/g | C/N Ratio | Dry Weight kg | BOD % | Absolute BOD kg |
|---|---|---|---|---|---|
| 0.00 | 438.60 | 57.02 | 14.00 | 38.68 | 5.42 |
| 4.00 | 429.90 | 56.17 | 13.97 | 38.55 | 5.39 |
| 8.00 | 416.90 | 53.46 | 13.83 | 37.13 | 5.14 |
| 12.00 | 404.20 | 50.02 | 13.62 | 35.20 | 4.79 |
| 16.00 | 392.60 | 45.83 | 13.16 | 32.66 | 4.30 |
| 20.00 | 380.70 | 41.37 | 12.83 | 29.40 | 3.77 |
| 24.00 | 357.50 | 36.04 | 12.46 | 23.95 | 2.98 |
| 28.00 | 329.00 | 30.86 | 12.04 | 18.98 | 2.29 |
| 32.00 | 285.10 | 27.47 | 11.59 | 16.67 | 1.39 |
| 36.00 | 228.10 | 25.57 | 11.20 | 15.47 | 1.73 |
| 40.00 | 120.40 | 22.88 | 10.78 | 14.70 | 1.59 |
| 44.00 | 70.20 | 21.33 | 10.33 | 13.19 | 1.36 |
| 48.00 | 57.00 | 20.47 | 10.27 | 12.80 | 1.31 |

* Moisture Content level kept 85 ± 5% through experiment period (Experimental lot)
** Agriculture Soil 36% + Sugarcane Bagasse Substrate 64 % by weight
*** Earthworm weight *Eudrilus eugeniae* initial weight 150 gm at the beginning of experiment
**** Earthworm weight *Eudrilus eugeniae* final weight 201 gm at the end of experiment i.e. 34% increase in weight


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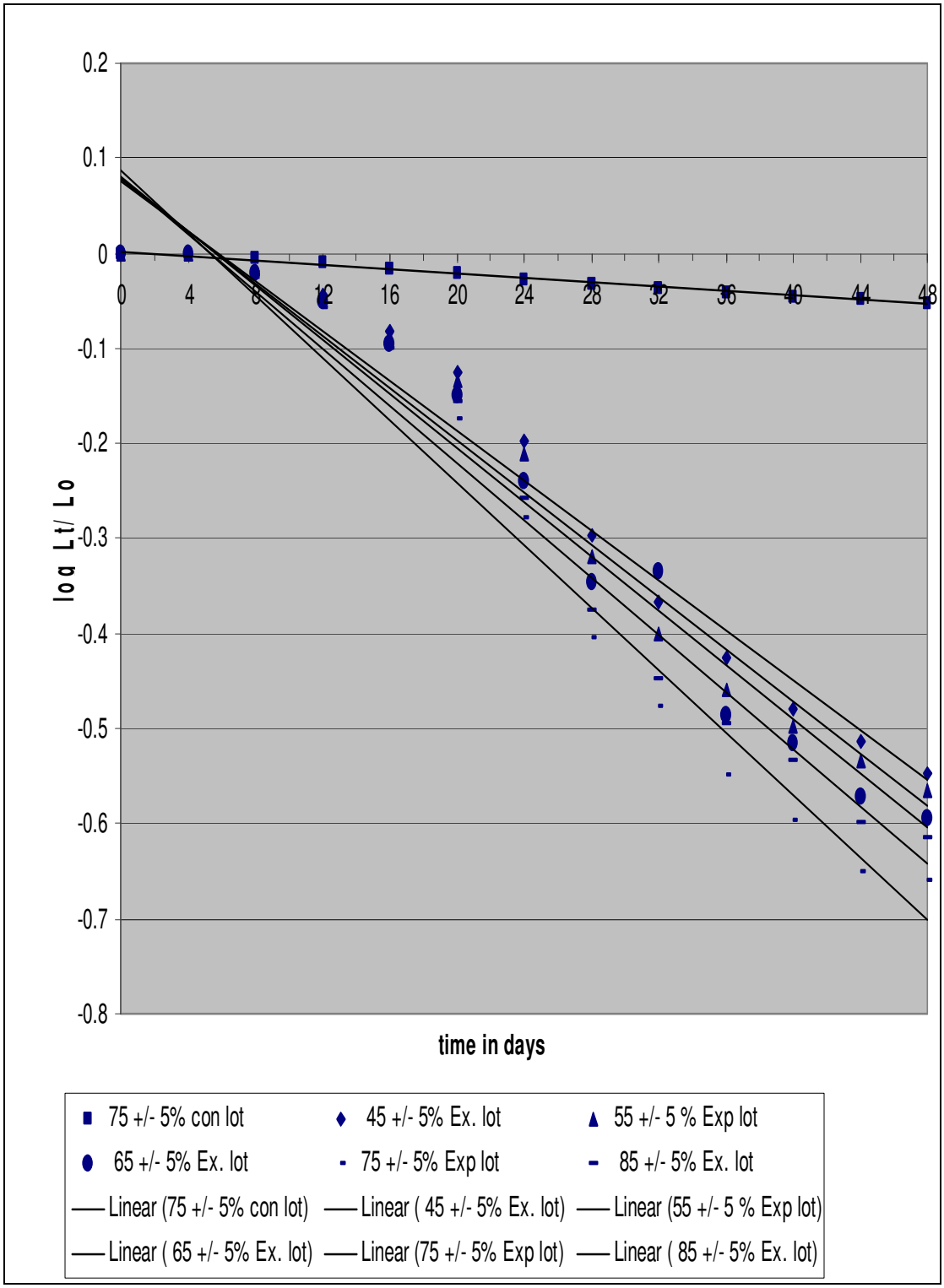


***Figure 1.*** *Reaction rate*


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## Discussion

Vermicomposting has become increasingly popular in the last two decades as an alternative to incineration of decomposable organic waste. In the whole process typical biochemical reactions involve were based on the path of bioconversion selected. In the current study the specie of earthworm *Eudrilus eugeniae* and variation in moisture content highly influenced the bioconversion process as displayed in table No. 2 to 6. Increased level of moisture content from 45± 5 % to 75± 5 % increases bioconversion and as the level reaches 85± 5 % bioconversion decreases. The present study points out that whenever moisture content level exceeds to 75± 5 % adverse effect are produced over bioconversion rate probably due to the replacement of existing voids of air by water which also results in emission of pungent odour [2] Further the water can also exert suction representing a negative pressure and the moisture retention can be termed as matric potential [23]. However in present study the matric potential has not been measured.

In cellulosic material i.e. sugarcane bagasse holds water in the cell wall which is dried at beginning of experiment. Present authors agree with Miller [35 & 36] that there is generation of high amount of metabolic water by hydrolysis of polysaccharides contributing to the total moisture content. Cellulose is a problematic organic waste which impedes the biodegradation process. Cellulose breakdown calls for a prolonged pretreatment as it contains several components like cellulose, hemicellulose, lignocelluloses, lignin, protein and fats. Authors agree with statement of Kyriaeou et.al [33] that during bioconversion enzyme complex secreted by microbial agencies present in experiment system, also causes the cellulose fragmentation activity. Kurakake et.al. [32] removed 76% of lignin by pretreatment of bagasse waste by cyclohexanol mixture. While Raul et. al. [38] did not give any degree of treatment to bagasse before their bioconversion study and measured Total Nitrogen, Cellulose, Hemicellulose, Lignin and Ash Content, Kurakake et.al [32] suggested that enzymatic hydrolysis process pretreatment causes hydrophobic degradation. In the present study no pretreatment technology was used. Only substrate bagasse and agriculture soil were dried in oven to remove the moisture held in both to help dry the cell wall of microorganisms present in soil. Vermicomposting is a mixed adventure caused by microorganisms and earthworms. To determine reaction rate at initial stage, soil and substrate were dried for natural growth of microflora and to estimate real reaction rate from beginning of experiment. Although the interactions between microorganisms and earthworms have received a considerable attention [6], the role of microorganism in gut transit is still controversial [51], even though it is proved now that the bacteria and fungi are digested by earthworms [18, 24 & 48]. During vermicomposting Doube and Brown [13] concluded that earthworm have minimum capacity to digest the cellulose containing organic residue and obtained their food nutrition by digestion of microbial flora, associated with ingested organic matter, which is only possible at optimum moisture content level.

### *Cellulose reduction*

In the present study of bagasse vermicomposting maximum percentage cellulose consumption was noticed at 75± 5 % moisture content level. It indicates that optimal biological activity takes place to degrade and stabilize the cellulose present in bagasse substrate with high metabolic biological reaction, hydrolysis and fragmentation


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occurring at certain level of moisture (Table 5). In the study of bioconversion, during composting of domestic refuse Kapetonios et.al. [30] recorded a faster degradation of cellulose than lignin. Godder and Peninck [20] recorded only 21.2 % cellulose reduction after 50 days of their experimentation and Kapteonios et.al. [30] have reported up to 53 % reduction of cellulose. Present authors recorded higher percentage cellulose reduction at lower duration compare to others.

### *C/N reduction*

C/N ratio is one of the most widely used parameter to monitor the progress of bioconversion. Much importance was given to this parameter by Raul et.al. [38], Flintoff [19], Tchobanoglous et.al. [47], Ambrose [2] and others. Sunderrajan et.al. [46] in their study during anaerobic digestion of kitchen waste did not consider C/N as a significant parameter to measure process, but chose COD and volatile solids to monitor the process development. Chanyasak and Kubota [11], recommended ratio of Carbon/Organic Nitrogen comparatively more dependable than C/N ratio and suggested non dependability of C/N ratio in solid waste composting as indicator of composting maturity and concluded that it can not be used as an absolute indicator of compost maturity. But question rises for its importance in bioconversion probably due to interchanging pattern of carbon released by carbohydrate degradation and established ammonification process, making both carbon and nitrogen highly fluctuating. The authors agree with Carvalho et.al. [9] contention, that C/N ratio is undependable due to its lack of control on entire vermicomposting process. Due to this reason authors laid more trust on percentage BOD and Absolute BOD as they are more dependable. Beside these authors suggested new dependable monitoring parameter like reaction rate (Kr) values to monitor the rate of vermicomposting reaction. Raul et.al. [38] recommended the optimum C/N ratio range from 30 to 50; Flintoff [19] advised that the suitable ratio may range from 20 to 70 depending on available carbon. The authors suggest that if the C/N ratio is kept up to 60 at initial stage of experiment, it will promote bioconversion process and will not exert any disorder during vermicomposting. In the present study the initial C/N ratio kept as 57.18 reached 19.38 by the 48th day of experimentation, indicating the near completion of vermicomposting process. Ashbolt and Line [3] have also reported a strong Nitrogen relationship to C/N ratio which supports authors work.

The Biochemical Oxygen Demand BOD values are often recorded in river, pond, stream, effluent and sewage etc. This indicative parameter shows the pollution level and is generally referred to BOD5 values from landfill leaches but fails to estimate reaction rate of biodegradation. As per authors suggestion the estimation of BOD value is essential for computing reaction rate. The earlier authors did not estimate BOD values as essential parameter, probably due to long procedure involved in determination. Much more attention is required to establish reaction rate (Kr) as a indicative parameter to hold safe and more control over the whole vermicomposting process.

### *Conclusion*

Vermicomposting is a mixed adventure of mixed microbes and earthworms. From previous experiments [37, 41, 42 & 43] we gather maximum activities of various microbial and earthworm species at best suited optimum level of moisture content. A constant monitoring and hold on progress of the process is thus required. In the present study bioconversion is also influenced by maturity of substrate due to presence of


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varying concentration of cellulose, hemicellulose, lignocelluloses, lignin etc. Introduction of specific microbial species synthesizing set of cellulase enzymes hastens the process of vermicomposting.

Enzymatic degradation proceeding as exothermal reaction retards the activity of earthworm beyond limit of their thermal tolerance. This may sweep of earthworm population from vermicompost thus causing reduction of fixed nitrogen and potassium level. It is therefore essential that use of specific inoculum for rapid degradation and higher nutritive values demand for control of liberated heat. Further experimental studies are required to establish final reaction rate and fix desired concentration of essential elements for healthy plant growth.

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# PLANNING LENGTH OF LONG -TERM FIELD EXPERIMENTS THROUGH DECISION SUPPORT SYSTEMS – A CASE STUDY

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**Abstract.** Long-term field experiments are the conventional means for developing; evaluating and demonstrating site-specific land/water use plans. However, it has been often observed that at times they are unable to propose sustainable practices, when planned for shorter time durations (say 2-3 years) or become cost-ineffective and obsolete, when planned for longer time durations (say more than 15 years or so). Hence, what should be the ideal length of such long-term experiments has always been a debatable issue. The present study attempts to demonstrate application of one indigenously developed decision support system (DSS) for planning appropriate length of long term conjunctive water use experiments on a test salt affected farmer's field. Before application the proposed DSS was extensively validated on several farmers and controlled experimental fields in Haryana (India). Validation of DSS showed its potential to give realistic estimates of root zone soil salinity; sodicity and salt stress induced relative crop yield reductions under local resource management conditions. Long term impact assessment of varied conjunctive water use strategies, on the test farmer's field, with the so validated DSS showed that the time required for achieving stable crop yields could be treated as a good measure of the minimum length of such experiments. For the test (rice-wheat growing) farmer's field, this time ranged between 5.5 – 6.0 years. It was observed that at shorter time scale (i.e. 2 years), though application of 50% canal waters (CW) blended with 50% tube well waters (TW), was as productive and superior as cyclic applications of (2CW, 1TW, 1CW); (1CW, 1TW, 2CW) and (CW: TW) during wheat cropping season yet it could not maintain its superior performance at DSS proposed time duration of 6 years or more. Similarly irrigation practice of (4CW, 5TW) during rice cropping season, though beneficial at shorter time scale, was much inferior to the cyclic application of canal and tube well waters (i.e. CW: TW) at longer time scales (i.e. at ≥ 6 years). Hence, limiting the proposed long-term conjunctive water use experiment to the DSS proposed minimum time duration of 6 years lead to the selection of the most stable and sustainable irrigation practice(s) for the test farm.
**Keywords:** *sustainability, long term studies, environmental impact assessment, water use planning*

## Introduction

Maintenance of long-term productivity and environmental conservation are the pre-requisites for sustainable agriculture. This requires proper use of natural resources. Planning proper use of natural resources presents several challenges before a decision maker. Appropriate land and water use decisions require the ability of a decision maker to understand their long-term impacts on soil health and crop production. Long-term field experiments, lasting 2 to several years, are the conventional means for the development, evaluation and demonstration of potentially sustainable practices as they provide a primary source of information about agricultural sustainability as a function of time [19].

A long-term field experiment is considered to be the one in which the original treatments are repeated on the same plots, year after year, for many years [8]. The


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classical experiments at Rothamsted, England [14] is a typical example. Such experiments play an important role in understanding the complex interaction of plants, soils, climate and management problems, and their effects on sustainable crop production. Although such experiments are essential for developing (site-specific) suitable land/ water use plans yet when planned for shorter time durations (say 2-3 years) they are unable to propose sustainable practices. Further, when planned for longer time durations (say more than 15 years or so) they become cost-ineffective and obsolete due to changes in agricultural practices with time. Hence what should be the ideal length of such long-term field experiments has always been a debatable issue.

Well-validated decision support systems with potential to evaluate time required for achieving stable crop yields, under varied agricultural systems, can be very effective tools for planning length of long-term field experiments. A Decision Support System (DSS) is infact an integrated assembly of models, data, interpretive routines and other relevant information that efficiently processes input data, runs the models, and displays the results in an easy-to-interpret format [10]. It comprises a hardware and a software to assist decision-makers in comprehensively looking at the relatively unstructured and complex environmental problems, try out different solutions and visualize the probable (short as well as long-term) impacts of adopting the solution, all through a computer. For planning purposes, this ability to dynamically change information, forecast and perform sensitivity analysis is exteremely useful.

The present study attempts to demonstrate the application of one such indigenously developed decision support system, named IMPASSE [15], for planning length of long term conjunctive water use experiments on a (test) rice-wheat growing salt affected farmer's field in Haryana (India).

## Materials and methods

### *Proposed DSS*

IMPASSE (IMPact Assessment and management of Saline/ Sodic Environments) is a user-friendly field scale-DSS designed for managing saline/ sodic soils and waters in freely draining irrigated and rainfed agricultural lands. It comprises of a set of well established subroutines for assessing short / long term impacts of a range of (geo) hydrologic conditions, water management options and crop rotation schedules on root zone-soil salinity / sodicity build ups and crop yield reductions [15]. By selecting appropriate time criteria it can even generate crop-specific irrigation schedules.

Under the assumption of no capillary rise from deep ground water tables in freely draining soils and no contribution of salts by rainfall, the change in soil root zone salt content (i.e. $\Delta Z$ in dS/m -mm or meq/l -mm) in the proposed DSS is based on the following equation proposed by [28]:

$$\pm \Delta Z = \{IS_i - (1\text{-}f)\, DS_i - (fD/ W_{fc})\, Z_{t\text{-}\Delta t}\}/ \{1 + fD/ 2W_{fc}\} \qquad \text{(Eq. 1.)}$$

While change in root zone soil moisture content ($\Delta W$ in mm) in a given time step $\Delta t$ (= 1 day) is expressed as:

$$(\pm)\ \Delta W = (I + P) - (E_a \text{ or } ET_a + R + D)$$

Where, $Z_{t\text{-}\Delta t}$ is initial salt content (in dS/m-mm or meq/l-mm) of soil root zone, $S_i$ is salt concentration (in dS/m) or $Na^+$, $Ca^{2+}$, $Mg^{2+}$ concentration (in meq/l) of irrigation water, 'f' is leaching fraction, $W_{fc}$ is field capacity moisture content (mm), I is the amount of


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applied irrigation water (mm), D is deep percolation loss (mm), P is precipitation (mm), $E_a$ is actual evaporation (mm) under fallow conditions, $ET_a$ (= $E_a$ + $T_a$) is actual evapo-transpiration (mm) under cropped conditions and R is surface runoff (mm). In the proposed DSS, daily actual evaporation and actual evapo-transpiration rates are computed through "square-root-of-time relation" [26] and Doorenbos and Pruitt [7] methods respectively.

Estimation of deep percolation loss (D) from soil root zone, under fallow/ upland conditions, is based on the following procedures:

$$D = \{(WC_t - WC_{fc}) / 100\} * RZD \text{ When } WC_{sat} > WC_t > WC_{fc} \text{ or}$$

$$D = \{(WC_{sat} - WC_{fc}) / 100\} * RZD \text{ When } WC_{sat} \leq WC_t > WC_{fc} \text{ or}$$

$$D = 0 \text{ When } WC_t = WC_{fc}$$

Where, $WC_{sat}$ is root zone soil moisture contents at saturation (mm), RZD is root zone soil depth (mm) and $WC_t$ is root zone soil moisture content (mm) at a particular time 't'.

For deep percolation losses under lowland (puddle) conditions, soil root zone is assumed to comprise of both the muddy layer and plow sole (responsible for impeded drainage) and the un-puddle soil, characterized with actual soil drainage rate [29]. Under such conditions, deep percolation is assumed to occur only when ponded water depth (Pond, in mm) is greater than zero. Ponding of water on the soil surface is assumed to take place only when root zone soil moisture at a particular time 't' ($WC_t$) is either greater than or equal to soil moisture content at saturation ($WC_{sat}$). When $WC_t > WC_{sat}$ then change in ponding depth in a given time interval $\Delta t$ (= 1 day) i.e. $\Delta$Pond (in mm) is set to $\{((WC_t - WC_{sat}) / 100) * RZD\} + P + I$. While, when $WC_t = WC_{sat}$ then $\Delta Pond = P + I$. Thus on days with Pond > 0, deep percolation losses under lowland/ puddle conditions are computed as follows:

$$\text{If Pond} \geq (K_s/ 5) \text{ then } D = (K_s/ 5) \text{ Else } D = \text{Pond}$$

Setting up of deep percolation losses (D) to $1/5^{th}$ of the saturated hydraulic conductivity values ($K_s$, in mm/day) in the above equation is in fact based on the findings of Fujioka [9] and Van de Goor [27], who observed that soil percolation rates of puddle soils are about $1/5^{th}$ of the same un-puddle soils.

Daily amounts of surface runoff (R) in the proposed DSS, depending upon upland/ fallow or lowland conditions, are computed as:

$$R = (I - \text{Bund}) \text{ When } I > \text{Bund, or } R = (P - \text{Bund}) \text{ When } P > \text{Bund, or}$$

$$R = (\text{Pond} - \text{Bund}) \text{ When Pond} > \text{Bund}$$

Where, 'Bund' is field bund depth (in mm) and 'Pond' is ponded water depth (in mm) under lowland conditions.

The so computed final root zone soil saturation extract-salt concentrations, obtained through Eq. 1, were adjusted for ion pair/ complex formation as per Sposito and Mattigod [25] method and expressed as Exchangeable Sodium Percentage (ESP, [4]) and Electrical Conductivity (EC) values.

Mechanistic simulation of salt stress induced crop yield reductions requires realistic information on root growth and water/ nutrient uptake distribution patterns, under varying salt stresses, for different crops [5]. However, non-availability of such (crop and location specific) information generally limits wide-scale applicability of these


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mechanistic approaches. Hence in the proposed DSS, daily relative crop yield reductions due to salinity ($EC_i$) or sodicity ($ESP_i$) stresses (i.e. $RYR_{si}$ and $RYR_{ai}$, respectively) are estimated through the following widely applicable empirical simulation procedure of Maas et al. [20]:

$$RYR_{si} = S_{ec} * (EC_i - EC_t)$$

$$RYR_{ai} = S_{esp} * (ESP_i - ESP_t) \quad \text{(Eq. 2.)}$$

While, daily (i.e. $RYR_{cdi}$) and seasonal (i.e. $RYR_{cg}$) relative crop yield reductions due to both salinity and sodicity stresses are expressed as:

$$RYR_{cdi} = MAX\ (RYR_{si}, RYR_{ai})$$

$$RYR_{cg} = MAX\ (RYR_{cd1}, RYR_{cd2}, \ldots\ldots, RYR_{cdi}) \quad \text{Eq. 3.)}$$

Where, $EC_t$ and $ESP_t$ are threshold salinity / sodicity levels, and $S_{ec}$ and $S_{esp}$ are slopes of salinity/ sodicity response functions for 30 different local crop types ranging from cereals, pulses, and oilseeds to vegetables [11, 12] .

The conceptualization of this salt transport and its impact on crop yield reductions is based on several theoretical assumptions such as: (1) no soil mineral dissolution, (2) no lateral/ upward capillary movement of water and salts, (3) incomplete mixing of root zone soil solution to account for bypass and (4) no direct crop yield reductions due to moisture, nutrient or pest stresses.

The proposed DSS was designed keeping in mind the relative simplicity of its operation to promote its use by field technicians and project planners. It contains a set of default soil/ crop characteristics, which can be selected and adjusted for various soil/ crop types. It basically requires two types of input-parameters. Type-I inputs comprise of daily weather data, crop data (viz. crop type and salinity/ sodicity response factors), soil data (viz. soil type, moisture contents at saturation, field capacity and wilting point, saturated hydraulic conductivity, initial soil moisture content and initial EC, $Na^+$, $Ca^{+2}$, $Mg^{+2}$ concentrations of soil root zone) and water data (viz. irrigation depth, application dates and EC and $Na^+$, $Ca^{+2}$, $Mg^{+2}$ concentrations). These parameters can be determined through actual resource surveys. While type-II inputs comprising of leaching fractions, whose direct determination is generally very cumbersome, are determined through calibration procedure.

### *DSS calibration & validation*

The proposed DSS was validated on several farmers as well as controlled experimental fields in Gurgaon and Karnal districts of Haryana, India. Before validation, firstly the calibrated values of leaching fractions under both actual farmers fields and controlled experimental field conditions were obtained. For this, soil type specific-default leaching fraction values [23] in the DSS were increased or decreased in steps till good correlation coefficients (R) between the observed and simulated EC and ESP values were obtained. The so calibrated leaching fraction values, along with the other type-I parameters, were then used for its validation. As per the recommendations of ASCE [2], both visual (graphical) and statistical comparisons in terms of correlation coefficient, mean relative error and root mean square prediction difference were used for this purpose.


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*On farmer's fields*

For validation on actual farmer's fields, a detailed inventory on weather, farming practices, soils and waters of 11-farmer's fields was prepared. Figure 1 illustrates the location of these fields in 6-villages of Gurgaon district of Haryana, India.

Test area weather data was acquired from Indian Meteorological Department (IMD) while farming practice information on crops cultivated, their sowing/ harvest dates, actual/ potential yields and water management practices was obtained through personal interviews of farmers.

Physico-chemical characteristics of the soils and waters of the test fields were determined through primary (farm-level) surveys, scheduled during the *Kharif* (June to September) and *Rabi* (October to March) seasons of 2000-03. During these surveys, bulk soil samples (1 Kg) from the top (0-30 cm) and the sub-soil (30-60 cm) horizons of 4 random spots, in each test farm, were extracted and mixed together to form one-composite top/ sub-soil sample. These composite soil samples were crushed and ground with a wooden mortar-pestle, passed through a 2-mm sieve and divided into three replicates. Simultaneously, bulk (250 ml) surface (i.e. Canal, C) and sub-surface (i.e. Tube Well, TW) water samples were also collected in labeled plastic bottles and partitioned into three equal portions. EC, pH, calcium, magnesium, sodium [13] and carbonate and bi-carbonate concentrations [22] of these (triplicate) soil/ water samples from each test farm were determined, as per the standard laboratory procedures. The so determined sodium, calcium, magnesium, carbonate and bi-carbonate concentrations in the soil saturation extracts and/ or water samples were then transformed to Sodium Adsorption Ratio (SAR), Exchangeable Sodium Percentage (ESP; [4]) and Residual Sodium Carbonate (RSC) values (meq/l), as per the standard procedures. Saturated hydraulic conductivities of non-destructive soil core samples [17], soil texture [3] and volumetric soil moisture contents at 0.05, 0.33 and 15 bars [16] were also determined.

The so determined actual (local) resource management conditions for the *Kharif* and *Rabi* seasons of 2000-01 were used for the proposed DSS-calibration while those for 2001-02 and 2002-03 seasons were used for its validation on 11- farmer's fields.


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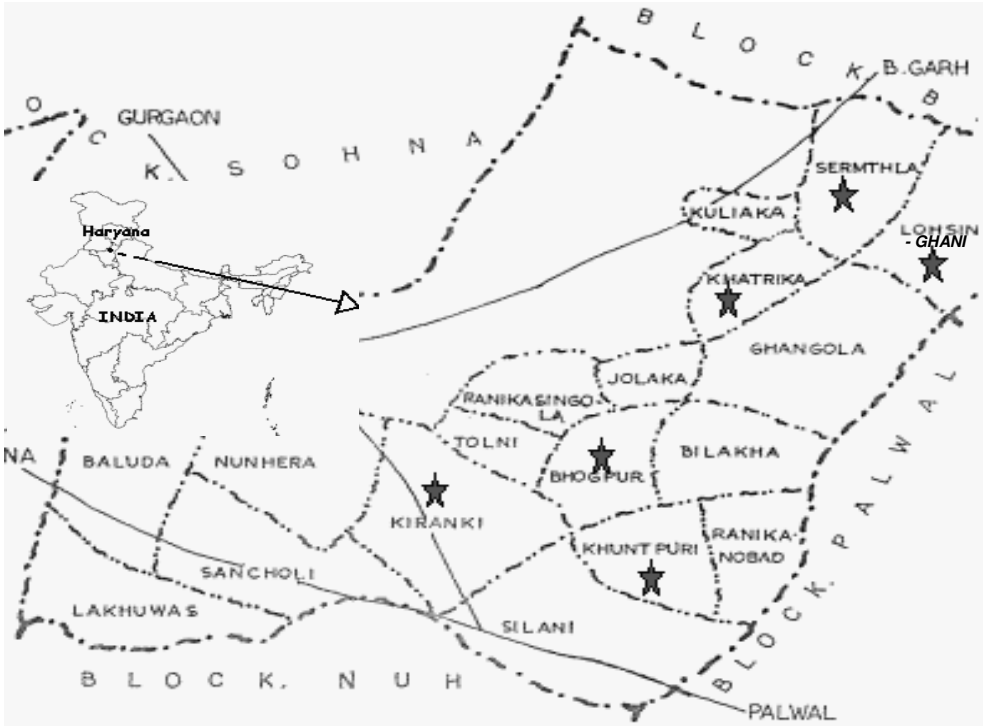


***Figure 1.*** *Location of test farms in Sohna block of Gurgaon district, Haryana (India)*

*On controlled experimental fields*

For this controlled (randomized block) experimental field data of Sharma et al. [24] was used. This experiment under fixed Wheat-fallow rotation (Nov., 1986 to Apr., 1989) comprised of 5-replications of 5-irrigation treatments viz. EC = 0.7 dS/m (Canal water, CW), 6 dS/m, 9 dS/m, 12 dS/m and 18 – 27 dS/m (Drainage water, DW). The first year (i.e. 1986-1987) experimental data was used for the calibration of the proposed DSS while the remaining 2-years data (for 1987-89 period) was used for its validation. For this study, actual soil salinization (EC) and economic yields under only first four irrigation treatments were considered. The economic yields obtained under 6, 9 and 12 dS/m - saline water irrigation treatments were divided with those under canal water irrigation (i.e. no salt stress) treatment to obtain actual relative crop yield reductions under varying salt-stress levels.

## *DSS application*

The so validated-DSS was then used for planning length of varied conjunctive water use experiments on a salt affected farmer's field in Khatrika village of district Gurgaon, Haryana (India). For this, long-term (10 years) impacts of varied (existing/ alternative) conjunctive water use strategies on the test field's root zone soil salinization/ sodification and relative crop yield reductions were simulated with the proposed DSS. While simulating long-term impacts of these conjunctive water use strategies, the test field was subjected to the long-term weather conditions (for the years 1990 – 1999) of the study area.

The test field, with Rice-Wheat cropping sequence, was characterized with cyclic application of good quality canal (EC: 0.9 dS/m, SAR: 3.1 and RSC: 0.6 meq/l) and


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poor quality tube well waters (EC: 4.3 dS/m; SAR: 12.7 and RSC: 4.1 meq/l). Existing conjunctive water use practice during *Kharif* (i.e. Rice crop) season comprised of 9-irrigations comprising of (3-TW, 3-CW and 3-TW). While that during *Rabi* (i.e. Wheat crop) season comprised of 4-irrigations with (2-CW, 1-TW, and 1-CW). Alternative (*Kharif* and *Rabi* season) conjunctive water use strategies simulated by the proposed DSS comprised of:

- *Kharif* season irrigation treatments*: 4-cyclic modes* i.e. (1-CW, 1-TW, 1-CW, 1-TW, 1-CW, 1- TW, 1-CW, 1-TW, 1-CW i.e. CW: TW); (1-TW, 1-CW, 1-TW, 1-CW, 1-TW, 1-CW, 1-TW, 1-CW, 1-TW i.e. TW: CW); (4-CW, 5-TW); (1-CW, 2-TW, 3-CW, 3-TW) and *2-blending modes* i.e. (50%CW + 50%TW i.e. 1:1) and (25%CW + 75%TW i.e. 1:3).

- *Rabi* season irrigation treatments: *3-cyclic modes* i.e. (1-CW, 1-TW, 2-CW); (2-CW, 2TW); (1-CW, 1-TW, 1-CW, 1-TW i.e. CW: TW); and *3-blending modes* i.e. (25%CW + 75%TW i.e. 1:3); (50%CW + 50%TW i.e. 1:1) and (75%CW + 25%TW i.e. 3:1).

While assessing long-term impacts of any conjunctive water use strategy during a particular season, the water use strategy for the subsequent season was kept as that existing for the test farm.

The so simulated long-term relative crop yield reductions under above (existing/ alternative) conjunctive water use strategies for *Kharif* and *Rabi* seasons were then subjected to a one way classified ANOVA analysis [6]. During this analysis, simulation years were assumed as classificatory variables while conjunctive water use strategies for a particular cropping season were assumed as more than one observation for a particular simulation year. This was basically aimed at determining the number of years in which a set of conjunctive water use strategies (or treatments) for a particular cropping season (or agricultural system) became sustainable.

## Results and discussions

### *DSS validation on actual farmer's fields*

The proposed DSS was calibrated and validated on 11-actual farmer's fields in 6-villages of Gurgaon district of Haryana state in India (Fig. 1). The study area experiences sub-tropical, semi-arid, continental and monsoonal type of climate. Paddy-Wheat in Lohsinghani, Sermethla, Khatrika, Bhogpur villages and Fallow-Wheat/ Barley in Kiranki-Kherli and Khuntpuri villages were the dominant crop rotation practices for the study area (Table 1). All test farms, excepting the one with no canal water supplies in Bhogpur village, used both canal and tube well waters for irrigation.


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***Table 1.*** *Local crop/water management practices for test farmer's fields*

| Village/ Farm | LOHSINGHANI | | SERMETHLA | | KHATRIKA | | *KHUNTPURI* | | BHOGPUR | KIRANKI-KHERLI | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Farm 1 | Farm 2 | Farm 1 | Farm 2 | Farm 1 | Farm 2 | Farm 1 | Farm 2 | Farm 1 | Farm 1 | Farm 2 |
| **Area (ha)** | 0.16 | 0.20 | 0.60 | 0.40 | 0.60 | 0.10 | 0.60 | 0.40 | 0.16 | 0.20 | 0.80 |
| **RABI SEASON** | | | | | | | | | | | |
| **Crop** | Wheat | Wheat | Wheat | Wheat | Wheat | Wheat | Barley | Wheat | Wheat | Wheat | Wheat |
| **Irrigation Source** | CW & TW | CW & TW | CW & TW | TW | CW & TW | CW & TW | TW | CW | TW | CW | CW & TW |
| **Irrigation No.** | 5 | 5 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 5 | 4 |
| **Irrigation Sequence** | 2CW,TW, CW, TW | 2CW,TW, CW, TW | 2TW, CW, TW | 4TW | 2CW,TW, CW | CW, TW, 2CW | 4TW | 4CW | 4TW | 4CW, TW | 4CW |
| **KHARIF SEASON** | | | | | | | | | | | |
| **Crop** | Paddy | Paddy | Paddy | Paddy | Paddy | Paddy | Fallow | Fallow | Paddy | Fallow | Fallow |
| **Irrigation Source** | CW & TW | CW & TW | CW & TW | TW | CW & TW | CW & TW | N/A | N/A | TW | N/A | N/A |
| **Irrigation No.** | 9 | 9 | 9 | 9 | 9 | 9 | N/A | N/A | 10 | N/A | N/A |
| **Irrigation Sequence** | 2CW, 2TW, 3C W, 2TW | 3CW, 2TW, 4CW | 3TW, 2CW, 4TW | 9TW | 3TW, 3CW, 3TW | 1CW, 1TW, 2CW, 3TW, 2CW | N/A | N/A | 10TW | N/A | N/A |


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Soils of farms in Lohsinghani, Khuntpuri, Bhogpur and Kiranki-Kherli villages were of sandy loam texture while those in Sermethla and Khatrika villages were of sandy clay loam texture. Their bulk density and gravimetric moisture contents at saturation, field capacity and wilting point ranged between 1.4 - 1.8 g/cm$^3$ and 29 - 39%; 16 to 24% and 7 to 11%, respectively. About 50% of the test farms were salt affected. Khuntpuri and Khatrika farms (with ESP: 33 -14 % and EC: 7- 4 dS/m) were observed to be the most salt-affected. Sermethla and Kiranki-Kherli farms (with ESP: 20-17% and EC: 3 dS/m) were moderately salt-affected while Lohsinghani and Bhogpur farms (with ESP: 15-10% and EC: 1-3 dS/m) were marginally salt affected. Sodicity was observed to be the major problem in the test farms.

Quality of canal waters of the study area was in general good (EC: 0.5-1.1 dS/m, pH: 6.8-8.4; SAR: 2.0-4.3 and RSC: 0.0-5.5 meq/l) while that of the tube-well waters was poor. Tube well waters of the test farms in Kiranki-Kherli and Khatrika villages were of the poorest quality (EC: 3.8-4.9 dS/m; SAR: 6.6-24.4 and RSC: 0.0-4.3 meq/l). These were followed by the tube-well waters in Khuntpuri (with EC: 3.0 dS/m; SAR: 4.1 and RSC: 3.0 meq/l); Lohsinghani (with EC: 1.4-2.1 dS/m; SAR: 3.2-16.2 and RSC: 0.0-3.9 meq/l); Bhogpur (with EC: 1.1-1.9 dS/m; SAR: 6.6-8.0 and RSC: 0.4-3.9 meq/l) and Sermethla (with EC: 0.8-1.4 dS/m; SAR: 2.0-15.4 and RSC: 0.0-3.8 meq/l) villages.

This information on actual local resource management conditions of 11-test farms, during *Kharif* and *Rabi* seasons of 2000-01, was used for the calibration of soil leaching fraction values in the proposed DSS. It was observed that the test farms with lowland paddy cultivation were associated with lower leaching fraction (f) values (ranging from 0.02 – 0.04) than the fallow (with 'f' ranging between 0.35 – 0.40) and Wheat/ Barley cultivated farms (with 'f' ranging between 0.20 – 0.40). Figure 2 shows that the so calibrated soil leaching fraction values (f, ranging between 0.02 - 0.40) could yield good correlation coefficients between the observed and simulated EC (0.96) and ESP (0.99) values of the test farms. Incorporation of these (calibrated) leaching fraction values, along with other (actually determined) type-I parameters for the test farms, into the proposed DSS resulted into EC and ESP predictions with Pearson correlation coefficients (R), absolute mean relative errors (AMRE) and root mean square prediction errors (RMSPD) ranging between 0.94 – 0.99; 0.02-0.06 and 0.51-0.84 respectively (Fig. 3) for the validation period (2001-03). It was observed that these root mean square prediction errors were in general lesser than the standard (measurement) errors (STD) associated with their observed EC and ESP values (EC_STD: 0.21- 1.76 dS/m and ESP_STD: 0.40-2.94%). Even the temporal profiles of soil root zone EC and ESP, for all test farms, could be quite realistically simulated by the proposed DSS.

### *DSS validation on controlled experimental fields*

Besides actual farmer's fields, the proposed DSS was calibrated and validated on several controlled experimental fields in Sampla experimental station of CSSRI, Karnal also. The soils of controlled experimental fields were of sandy loam texture with bulk density and gravimetric moisture contents at field capacity and saturation ranging between 1.48 -1.55 g/cm$^3$, 8 - 21 % and 35 - 41 % respectively. Initial root zone (0-60 cm) soil salinity of these experimental fields (before Wheat crop sowing in Nov. 1986) ranged between 1.5 – 1.9 dS/m.


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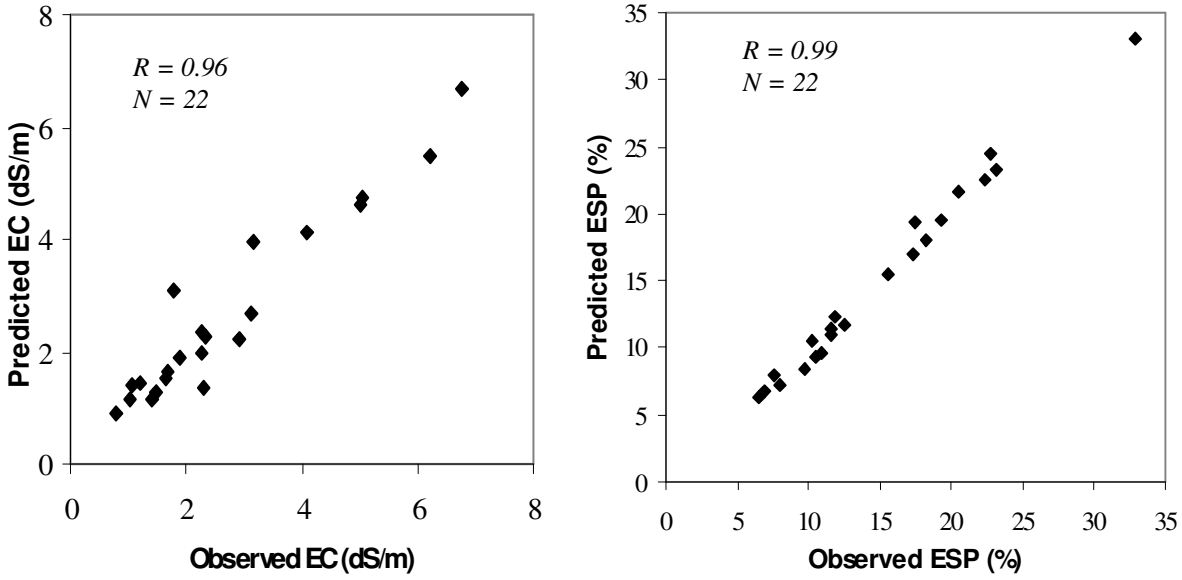


***Figure 1.*** *Observed vs. predicted EC and ESP values for farmer's fields during (2000-01) calibration period*

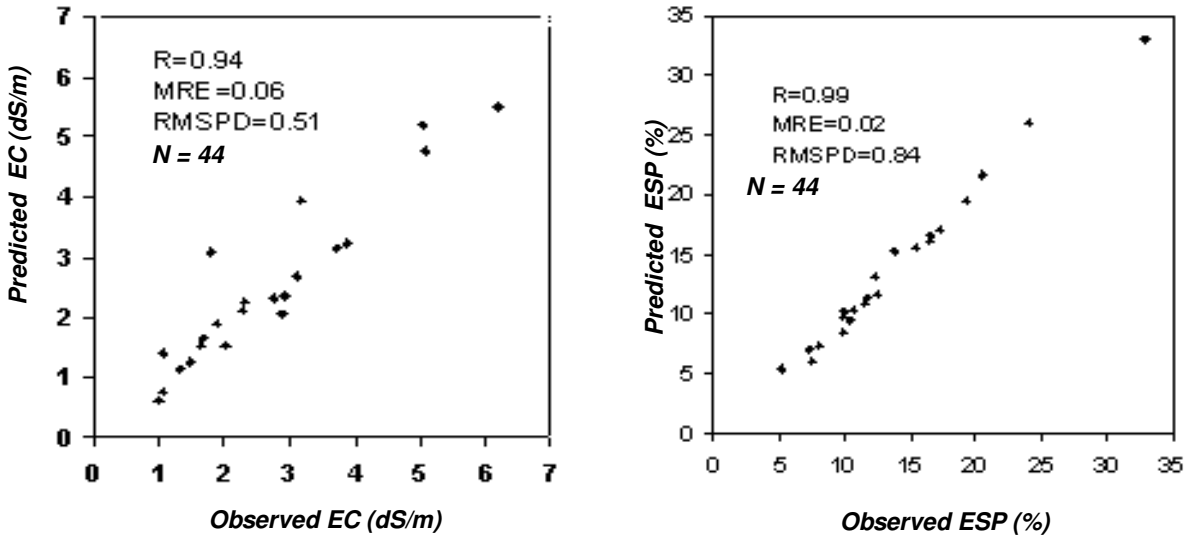


***Figure 2.*** *Overall correlation coefficients (R), mean relative errors (MRE) and root mean square prediction errors (RMSPD) for DSS simulated EC and ESP values for farmer's fields during (2001-03) validation period*

Calibration of proposed DSS on the controlled experimental field data for 1986-1987 showed (Fig. 4) that soil leaching fraction value of 0.5 could yield reasonably good correlation coefficient (0.86) between the observed and predicted EC values for the test fields. Incorporation of these calibrated leaching fraction values, along with other (known) type-I parameters, into the proposed DSS showed that simulated root zone electrical conductivities (under 4-saline water irrigation treatments) for (1987-89)


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validation period were associated with correlation coefficients (R), and root mean square prediction errors (RMSPD) ranging between 0.84 - 0.96 and 0.42 - 1.30 respectively (Table 2). Pooling of validation results for all 4 - irrigation treatments showed that absolute mean relative errors associated with the DSS-predicted root zone soil salinities were well within 15%.

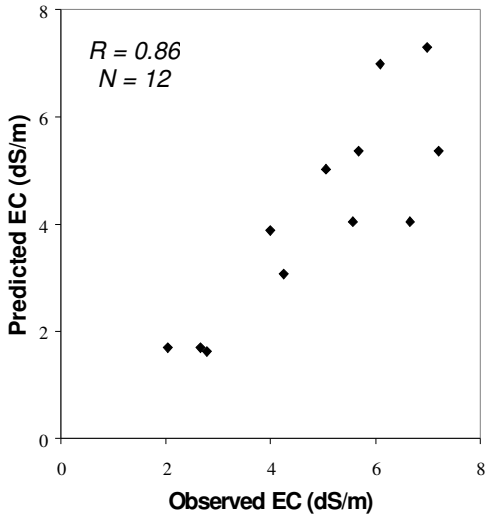


***Figure 3.*** *Observed vs. predicted electrical conductivities (EC) of controlled experimental fields during (1986-87) calibration period*

***Table 2.*** *Observed (Obs.) vs. DSS-predicted (Pred.) electrical conductivities (EC, dS/m) of controlled experimental fields during validation period (1987-89)*

| Dates | CW (< 0.7 dS/m) | | 6(dS/m) | | 9 (dS/m) | | 12 (dS/m) | |
|---|---|---|---|---|---|---|---|---|
| | Obs. | Pred. | Obs. | Pred. | Obs. | Pred. | Obs. | Pred. |
| 13/11/87 | 1.50 | 1.46 | 2.75 | 3.33 | 4.15 | 4.37 | 5.00 | 5.66 |
| 10/02/88 | 1.59 | 1.49 | 2.95 | 4.13 | 4.25 | 5.61 | 5.97 | 7.32 |
| 09/03/88 | 2.05 | 1.46 | 4.61 | 4.7 | 6.23 | 6.51 | 8.35 | 8.54 |
| 24/03/88 | 1.87 | 1.44 | 5.20 | 5.25 | 6.56 | 7.38 | 8.47 | 9.72 |
| 09/04/88 | 1.52 | 1.53 | 5.46 | 6.17 | 7.34 | 8.79 | 8.96 | 11.59 |
| 16/11/88 | 0.68 | 0.28 | 0.63 | 0.82 | 0.50 | 1.12 | 0.48 | 1.45 |
| 09/04/89 | 1.24 | 0.51 | 5.07 | 3.55 | 5.99 | 5.26 | 7.34 | 6.99 |
| **R** | 0.84 | | 0.88 | | 0.95 | | 0.96 | |
| **RMSPD** | 0.42 | | 0.81 | | 0.90 | | 1.30 | |

The above controlled experimental field data (for 1987-89 period) was also used for testing the proposed decision support system's potential to realistically simulate relative crop yield reductions under varying salinity levels. In contrast to the actual relative wheat yield reductions of about 5.94, 19.93 and 22.07%, under 6, 9 and 12 dS/m-irrigation treatments, the proposed DSS predicted about 6.25, 18.03 and 38.61% relative


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crop yield reductions respectively. These DSS simulated relative wheat yield reductions were associated with absolute mean relative error of 24%.

Hence validation of proposed DSS on both farmer's and controlled experimental fields showed that it was indeed capable of giving near-realistic estimates of both root zone soil salinity/ sodicity and relative crop yield reductions.

### *Assessing length of conjunctive water use experiments with DSS*

Average rice and wheat crop yield reductions, at initial mean salt concentrations of 5.2-dS/m (EC) and 16.0% (ESP) under varied conjunctive water use strategies, on the test farm were about 39% and 28% respectively. Long-term impacts of varied *Kharif* season-conjunctive water use strategies with the proposed DSS (Table 3) showed about 17% improvement in mean rice crop yields over 10 years duration. However it was observed that these average rice crop yields had no significant improvement beyond fourth year. On the contrary, mean wheat crop yield reductions (Table 4), under varied *Rabi* season-conjunctive water use strategies, stabilized at about 14% in the eighth year of the long-term impact assessment study. Hence, the proposed DSS could clearly show that the length of rice crop based long-term conjunctive water use experiments, associated with larger water inputs (Table 1) and hence faster leaching of root zone salts, should be shorter than the wheat crop based conjunctive water use experiments.

***Table 3.*** *DSS-simulated long-term impacts of varied Kharif season-conjunctive water use strategies on test farm's rice crop yield reductions*

| **Simulation year** | **Rice crop yield reductions (%) under individual conjunctive water use strategies** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CW: TW | TW: CW | 4CW, 5TW | 1CW, 2TW, 3CW, 3TW | 3TW, 3CW, 3TW **(Existing)** | 25%CW + 75%TW | 50%CW + 50%TW | MEAN |
| 1 | 38.1 | 38.4 | 42.1 | 38.4 | 38.4 | 39.9 | 38.1 | 39.1 (a)[#] |
| 2 | 29.0 | 32.7 | 30.2 | 33.2 | 33.9 | 34.6 | 26.6 | 31.5 (b) |
| 3 | 19.3 | 22.7 | 20.9 | 24.1 | 24.3 | 25.5 | 19.2 | 22.3 (d) |
| 4 | 20.4 | 23.2 | 23.2 | 25.1 | 25.3 | 27.5 | 20.9 | 23.7 (c, d) |
| 5 | 20.7 | 23.4 | 24.2 | 25.5 | 25.7 | 28.5 | 21.7 | 24.3 (c) |
| 6 | 20.7 | 23.3 | 24.8 | 25.8 | 25.8 | 28.9 | 22.1 | 24.5 (c) |
| 7 | 20.7 | 23.4 | 25.1 | 27.1 | 25.9 | 29.1 | 22.3 | 24.8 (c) |
| 8 | 19.2 | 22.0 | 27.1 | 26.7 | 27.2 | 24.1 | 20.9 | 23.9 (c) |
| 9 | 20.4 | 22.4 | 26.6 | 24.2 | 23.1 | 27.0 | 21.8 | 23.6 (c, d) |
| 10 | 20.4 | 22.4 | 26.0 | 26.8 | 24.5 | 28.1 | 21.7 | 24.3 (c) |

#: Means with the same letter are not significantly different at $\alpha$= 0.05 with Critical t-Value = 2.0 and Least Significant Difference = 1.4


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***Table 4.*** *DSS-simulated long-term impacts of varied Rabi season-conjunctive water use strategies on test farm's wheat crop yield reductions*

| Simulation year | Wheat crop yield reductions (%) under individual conjunctive water use strategies | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2CW, 1TW, 1CW (**Existing**) | 1CW, 1TW, 2CW | 2CW, 2TW | CW: TW | 25% CW + 75% TW | 50% CW + 50% TW | 75% CW + 25% TW | MEAN |
| 1 | 28.3 | 27.7 | 28.8 | 28.1 | 28.6 | 27.7 | 26.9 | 28.0 (a)[#] |
| 2 | 21.2 | 20.9 | 26.0 | 21.3 | 24.6 | 21.2 | 19.7 | 22.1 (b) |
| 3 | 18.1 | 18.4 | 22.5 | 18.2 | 21.7 | 21.2 | 18.1 | 19.7 (c) |
| 4 | 16.2 | 16.0 | 21.6 | 16.1 | 20.5 | 19.5 | 15.2 | 17.9 (d) |
| 5 | 15.1 | 14.8 | 19.2 | 15.0 | 19.5 | 18.2 | 14.6 | 16.6 (e) |
| 6 | 14.2 | 14.3 | 18.5 | 14.3 | 18.7 | 17.3 | 14.2 | 15.9 (e) |
| 7 | 13.4 | 13.5 | 17.3 | 13.4 | 17.9 | 17.1 | 13.0 | 15.1 (f) |
| 8 | 13.1 | 13.1 | 15.8 | 13.1 | 15.7 | 15.1 | 12.7 | 14.1 (g) |
| 9 | 13.1 | 12.5 | 14.9 | 12.9 | 15.4 | 14.2 | 12.0 | 13.5 (g) |
| 10 | 13.2 | 12.2 | 14.7 | 13.3 | 15.1 | 13.9 | 11.7 | 13.4 (g) |

#: Means with the same letter are not significantly different at $\alpha$= 0.05 with Critical t-Value = 2.0 and Least Significant Difference = 0.9

It was further observed that DSS proposed mean annual root zone salt concentrations of the rice-wheat growing test farm, exposed to varied conjunctive water use strategies, decreased exponentially with time (Fig. 5a, b). Fitting of these mean annual EC and ESP values to an exponential model showed that the test farm was associated with mean salt decay rates of -0.023 dS/m and -0.016% per annum at 95% confidence interval. It was estimated that at these decay rates, time required for the test farm's root zone EC/ ESP values to stabilize at (all treatment) mean values of 4.6 dS/m or 14.5 %, from an initial mean levels of about 5.2 dS/m or 16.0%, is about 5.5 to 6.0 years.

The present study could thus clearly show that the time required for achieving stable root zone salt concentrations, as revealed from stable crop productivities, under individual Rice / Wheat cropping seasons is quite different from that required by combined Rice-Wheat system. In fact in a combined Rice-Wheat system, the time required for achieving stable root zone salt concentrations was about average (i.e. 5.5 – 6.0 years) of both quickly (i.e. in about 4 years)-stabilizing rice and slowly (i.e. in about 8 years)- stabilizing wheat sub-system. These results were in complete confirmation with those obtained through actual long-term conjunctive water use experiments of All India Co-ordinated Research Projects [1] on Rice-Wheat cropping sequence. The proposed DSS could hence quite realistically mimic the natural tendency of a faster sub-system (i.e. Rice) to force the total (i.e. Rice-Wheat) system's stabilization time to lower than that of a slower sub-system (i.e. Wheat) and vice-a-versa.


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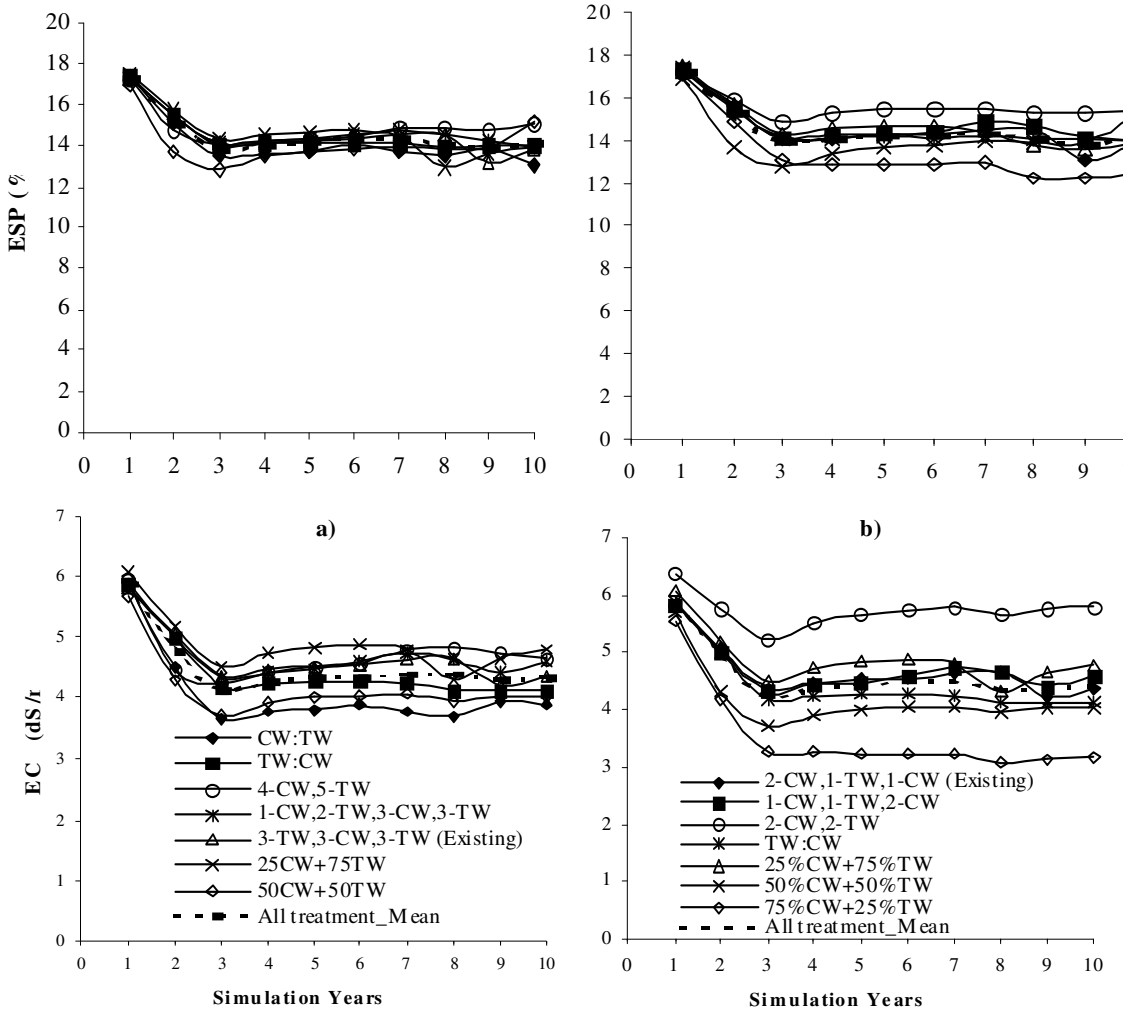


***Figure 4.*** *Long-term average annual root zone salt concentrations under (a) Kharif and (b) Rabi season - conjunctive water use strategies for the test farm*

Impact of length of long term experiments on the consistent performance and hence appropriate selection of most stable and sustainable conjunctive water use strategies for the test farm was also attempted. Table 3 shows that at shorter time scales (eg. 2 years) (50% CW + 50% TW); (CW:TW) and (4CW,5TW) conjunctive water use strategies appeared to be superior to the rest of the *Kharif* season-conjunctive water use strategies. However at DSS proposed stabilization time for the (test) Rice-Wheat system (i.e. 6 years) or at longer term scales, cyclic application of (4CW,5TW) appeared to be much inferior to (50% CW + 50% TW); (CW:TW) and (TW:CW) strategies. In fact it was observed that, at longer time scales (i.e. at ≥ 6 years), cyclic application of canal and tube well waters (i.e.CW:TW) was consistently more productive than anyother *Kharif* season conjunctive water use strategy for the test farm.

Similarly, on comparing various conjunctive water use strategies for *Rabi* season (Table 4), it was observed that although on shorter time scale (i.e. 2 years) application of 50% CW blended with 50% TW (i.e. 50% CW + 50% TW) was as productive and superior as cyclic applications of (2CW, 1TW, 1CW); (1CW, 1TW, 2CW) and


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(CW:TW) yet on long term scales (i.e. at ≥ 6 years) it could not maintain its superior performance. In fact, at longer time scales, (75%CW + 25% TW); (1-CW, 1-TW, 2-CW); (CW: TW) and (2-CW, 1-TW, 1-CW, i.e. existing) irrigation practices appeared to be the most stable and consistent strategies for the test farm. It was further observed that during any cropping season, cyclic conjunctive water use strategies were in general more (long-term) stable and sustainable than the blending options. These results were in complete confirmation with those obtained through several actual long-term conjunctive water use experiments [21, 18] on similar soils.

## Conclusions

The present investigation could demonstrate tremendous application potential of such pre-validated decision support tools in planning appropriate lengths of various long-term field experiments. It could clearly demonstrate that different agricultural systems, as generated by different sets of conjunctive water use treatments, are characterized with different time periods for achieving stable/sustained crop yields. Limiting an infinitely long conventional field experiment to this time duration not only leads to the selection of most appropriate and sustainable agricultural practice(s) but also increases its cost, time, energy and information-efficiency and hence chances to be planned for many other diverse locations within the same limited budget.

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# QUALITY OF GROUND WATER OF JAIPUR CITY, RAJASTHAN (INDIA) AND ITS SUITABILITY FOR DOMESTIC AND IRRIGATION PURPOSE

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**Abstract:** The groundwater quality of Jaipur city experienced degradation due to rapid urbanization and industrialization. The hydrochemical investigation in the present study is restricted to the major ions concentrations, distributions, their relative abundance, and the pattern of the variability in groundwater chemistry. On the basis of the groundwater chemistry an evaluation of groundwater for domestic and irrigation uses is established. Eleven ground water samples were collected from Jaipur City, Rajasthan (India) from different hand pumps to study the chemical parameter, such as pH, EC, Total Hardness, Calcium, Magnesium, Sodium, Potassium, Carbonate, Bicarbonate, Sulphate, and Chloride, with the help of standard method of APHA during pre-monsoon (April 2006 to June 2006).
**Keywords:** *Jaipur City, ground water, pre-monsoon, physico-chemical parameter, hydrochemistry*

## Introduction

Water is nature's most wonderful, abundant and useful compound and it is the basis of all lives–ecological resources for the flora and fauna of our earth and a fundamental necessity for all lives. Without a properly functioning water supply, it is difficult to imagine productive human activity, be it agriculture or forestry, livestock, farming or fisheries, trade or industry.

The quality of water is of upper most importance compared to quantity in any water supply planning and especially for drinking purposes purity is equally important. The chemical, physical and bacterial characteristics of ground water determine its usefulness for municipal, commercial, industrial, agricultural, and domestic water supplies[17].

Jaipur City (Longitude: 95°24' E; latitude: 27°18' N), the capital city of Rajasthan (INDIA) is one of the fastest growing cities in the country, is undergoing rapid urbanization and industrialization. Urbanization has led to immense pressure on ground water resources and has resulted in quality deterioration of ground water as well.

### *Review of literature*

Various workers in our country have carried out extensive studies on Water Quality. Abbasi [1] et al and Jagdap Jyashri [5] et al have studied water quality of different rivers. Shriniwas [14] et al and Jha [7] et al studied water quality in Hydrabad and Bihar, respectively. Patnaik [8] et al reported water pollution in industrial area. Fluoride level in drinking water from various sources in and around Jaipur and in many villages and trace metals have been carried out in our laboratory [6–11] earlier. Studies of industrial wastewater and ground water and pollution problem in ground water have


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also been studied in our laboratory [12–13] recently. The objective of the scientific investigations is to determine the hydrochemistry of the ground water and to classify the water in order to evaluate the water suitability for drinking, domestic and irrigation uses and its suitability for municipal, agricultural and industrial use.

### *Materials and methods*

Ground water samples from different hand pumps of eleven sampling points were analyzed during pre-monsoon (April, 2006 to June, 2006). Samples were collected in good quality polyethylene bottles of one-litre capacity. Sampling has been carried out without adding any preservatives in well-rinsed bottles. Only high pure (AnalR grade) chemicals and double distilled water was used for preparing solution for analysis.

Various physical parameters like pH, EC, and TDS were determined at the site with the help of digital portable water analyzer kit (CENTURY–CK–710). Calcium ($Ca^{2+}$), Magnesium ($Mg^{2+}$), Chloride ($Cl^{-}$), Carbonate ($CO_3^{2-}$) and Bicarbonate ($HCO_3^{-}$) and Sulphate ($SO_4^{2-}$) by volumetric titration methods; while Sodium ($Na^{+}$) and Potassium ($K^{+}$) by Flamephotometry (ELICO–CL–220) (APHA [2] et al, 1985). The sodium adsorption ratio (SAR) values of each water sample were calculated by using Richard [10] equation and the total hardness (TH) in ppm and the sodium percentage (Na%) values were determined using the equations Todd [15].

The respective values for all these parameters are reported in Table 1 and Table 2; all results are compared with standard limit recommended by the Bureau of Indian Standards (BIS), Indian Council of Medical Research (ICMR), and WHO.

## Results and discussion

The concentration of cations and anions are incorporated in Table 1. Classification of waters depends on the principle of the IAH (International Association of Hydrogeologist)[4], 1979. Total equivalents of cations and anions were taken as 100% and ions, as more than 20% (meq/L), were evaluated in the classification. Other Physico-chemical parameters are shown in Table 2. Physico-chemical properties of ground water of Jaipur City Compared to Standards are shown in Table 3.

### *Groundwater quality for drinking water purposes*

#### *pH*

It was observed from the pH value that water samples were varying from 7.40 to 8.03 and these values are within the limits prescribed by ISI, ICMR (Table 3) the ground water sample collected from J-13 show pH value of 9.78 that is higher than the permissible limit and indicates that water is slightly alkaline.

#### *Electrical Conductivity (EC)*

EC of the groundwater is varying from 345 to 2550 microsiemens/cm at 25°C .the maximum limit of EC in drinking water is prescribed as 1400 microsiemens/cm (WHO: 2003) Samples (J-10, J-14, J-17 and J-21) exceed the permissible limit.


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*Total Dissolving Solids (TDS)*

The total dissolved solids in water are represented by the weight of residue left when a water sample has been evaporated to dryness. TDS values varied from 239.6 to 1435 mg/l except J-6, J-12, J-13, J-24; all remaining samples exceed the permissible limit prescribed by WHO (500 mg/l) and ICMR (600 mg/l).

***Table 1.*** *Ionic variation in ground water of Jaipur City*

| Code | Unit | $Ca^{2+}$ | $Mg^{2+}$ | $Na^{+}$ | $K^{+}$ | $Co_3^{2-}$ | $HCO_3^{-}$ | $Cl^{-}$ | $So_4^{2-}$ | Water type |
|---|---|---|---|---|---|---|---|---|---|---|
| | ppm | 14.03 | 34.05 | 200 | 4.10 | 30 | 196 | 204.94 | 10 | Na-Mg-Cl-$HCO_3$ |
| J-1 | epm | 0.70 | 2.80 | 8.70 | 0.10 | 1.00 | 3.21 | 5.78 | 0.21 | |
| | % | 5.69 | 22.76 | 70.70 | 0.85 | 9.80 | 31.49 | 56.66 | 2.04 | |
| | ppm | 20.04 | 6.08 | 134 | 0.90 | 42 | 237.90 | 54.48 | 47.85 | Na-$HCO_3$ |
| J-6 | epm | 1.00 | 0.50 | 5.83 | 0.02 | 1.40 | 3.90 | 1.54 | 1.00 | |
| | % | 13.60 | 6.80 | 79.28 | 0.31 | 17.87 | 49.79 | 19.62 | 12.72 | |
| | ppm | 254.50 | 24.32 | 164 | 5.50 | ND | 396.50 | 624.81 | 112.50 | Ca-Na-Cl-$HCO_3$ |
| J-10 | epm | 12.70 | 2.00 | 7.13 | 0.14 | | 6.50 | 17.63 | 2.34 | |
| | % | 57.79 | 9.10 | 32.46 | 0.64 | | 24.56 | 66.59 | 8.85 | |
| | ppm | 30.06 | 36.48 | 37 | 0.60 | ND | 259.25 | 37.49 | 11.61 | Mg-Na-Ca-$HCO_3$ |
| J-12 | epm | 1.50 | 3.00 | 1.61 | 0.02 | | 4.25 | 1.06 | 0.24 | |
| | % | 24.49 | 48.98 | 26.28 | 0.25 | | 76.59 | 19.06 | 4.36 | |
| | ppm | 10.02 | 3.65 | 65 | 1.50 | 42 | 6.10 | 37.49 | 74.58 | Na-$SO_4$-$CO_3$-Cl |
| J-13 | epm | 0.50 | 0.30 | 2.83 | 0.04 | 1.40 | 0.10 | 1.06 | 1.55 | |
| | % | 13.64 | 8.19 | 77.13 | 1.05 | 34.06 | 2.43 | 25.73 | 37.78 | |
| | ppm | 64.13 | 107.01 | 93 | 3.20 | 6 | 207.40 | 227.43 | 104.40 | Mg-Na-Ca-Cl-$HCO_3$ |
| J-14 | epm | 3.20 | 8.80 | 4.05 | 0.08 | 0.20 | 3.40 | 6.42 | 2.17 | |
| | % | 19.84 | 54.57 | 25.08 | 0.51 | 1.64 | 27.89 | 52.63 | 17.83 | |
| | ppm | 130.26 | 126.46 | 54 | 2.80 | ND | 183 | 399.87 | 96.32 | Mg-Ca-Cl |
| J-17 | epm | 6.50 | 10.40 | 2.35 | 0.07 | | 3.00 | 11.28 | 2.01 | |
| | % | 33.64 | 53.83 | 12.16 | 0.37 | | 18.42 | 69.26 | 12.31 | |
| | ppm | 20.04 | 24.32 | 190 | 2.40 | 33 | 423.95 | 82.47 | 37.11 | Na-$HCO_3$-Cl |
| J-18 | epm | 1.00 | 2.00 | 8.26 | 0.06 | 1.10 | 6.95 | 2.33 | 0.77 | |
| | % | 8.83 | 17.66 | 72.97 | 0.54 | 9.87 | 62.34 | 20.87 | 6.93 | |
| | ppm | 30.06 | 79.04 | 271 | 2.30 | 15 | 503.25 | 179.94 | 60.46 | Na-Mg-$HCO_3$-Cl |
| J-19 | epm | 1.50 | 6.50 | 11.79 | 0.06 | 0.50 | 8.25 | 5.08 | 1.26 | |
| | % | 7.56 | 32.75 | 59.39 | 0.30 | 3.31 | 54.69 | 33.65 | 8.34 | |
| | ppm | 90.18 | 111.87 | 132 | 7.60 | 24 | 420.90 | 402 | 112.35 | Mg-Na-Ca-Cl-$HCO_3$ |
| J-21 | epm | 4.50 | 9.20 | 5.74 | 0.19 | 0.80 | 6.90 | 11.34 | 2.34 | |
| | % | 22.92 | 46.85 | 29.24 | 0.99 | 3.74 | 32.27 | 53.04 | 10.94 | |
| | ppm | 26.05 | 20.67 | 53 | 2.50 | 36 | 195.20 | 32.49 | 4.90 | Na-Mg-Ca-$HCO_3$ |
| J-24 | epm | 1.30 | 1.70 | 2.31 | 0.06 | 1.20 | 3.20 | 0.92 | 0.10 | |
| | % | 24.21 | 31.66 | 42.94 | 1.19 | 22.15 | 59.06 | 16.91 | 1.88 | |

*Calcium ($Ca^{2+}$)*

Calcium concentrations are varying from 10.02 to 254.50 mg/l and except J-10; all samples are within permissible limit prescribed by ICMR.


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***Table 2.*** *Physico-chemical parameters of groundwater of Jaipur city*

| CODE | pH | EC | TDS | TH | SAR | Na% |
|---|---|---|---|---|---|---|
| J-4 | 7.72 | 1149 | 611.50 | 175.15 | 6.58 | 71.55 |
| J-6 | 7.9 | 791 | 445.00 | 75.06 | 6.73 | 79.60 |
| J-10 | 7.4 | 2540 | 1435.14 | 735.56 | 2.63 | 33.10 |
| J-12 | 7.73 | 587 | 296.69 | 225.18 | 1.07 | 26.53 |
| J-13 | 9.78 | 345 | 239.46 | 40.04 | 4.47 | 78.17 |
| J-14 | 7.84 | 1596 | 785.64 | 600.48 | 1.65 | 25.59 |
| J-17 | 7.56 | 2550 | 982.57 | 845.64 | 0.81 | 12.53 |
| J-18 | 8.03 | 1130 | 654.79 | 150.12 | 6.75 | 73.51 |
| J-19 | 7.76 | 1704 | 961.63 | 400.31 | 5.89 | 59.69 |
| J-21 | 7.56 | 2430 | 1112.60 | 685.52 | 2.19 | 30.23 |
| J-24 | 7.93 | 540 | 275.85 | 150.10 | 1.88 | 43.80 |

*Magnesium ($Mg^{2+}$)*

Magnesium concentration varies from 3.65 to 126.46 mg/l and these values are within permissible limits prescribed by ISI, ICMR, WHO.

*Total Hardness (TH)*

In most water nearly all the hardness is due to calcium and magnesium. All the metallic cations besides the alkali metals also cause hardness. Total Hardness is varying from 40.04 to 845.64 mg/l and these values are within the permissible limit prescribe by ISI, ICMR and WHO except J-10, J-14, J-17, J-21.

*Sodium ($Na^+$) and Potassium ($K^+$)*

Large amounts give a salty taste when combined with chloride. Moderate quantities have little effect on the usefulness of water for most purposes. Sodium and potassium concentrations are varying from 20.67 to 200 mg/l and 0.6 to 7.6 mg/l respectively.

*Chloride ($Cl^-$)*

Chloride concentration are varying from 32.49 to 624.81 mg/l which are lower than the prescribed by ICMR and WHO standards except for J-17, J-21, J-10. In sample (J-10) chloride concentration is very high (624.81mg/l). Unusual Concentration may indicate pollution by organic waste.

Chloride salts in excess of 100 mg/1 give salty taste to water. When combined with calcium and magnesium, may increase the corrosive activity of water. It is recommended that chloride content should not exceed 250 mg/1.

*Carbonate (CO32-) and Bicarbonate ($HCO3^-$)*

Carbonate and Bicarbonate concentration are varying from 6 to 42 mg/l and 6.10 to 503.25 mg/l respectively.


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*Sulphate ($SO_4^{2-}$)*

Sulphate concentration is varying from 8.55 to 112.5 mg/l and these values are within permissible limits prescribed by ISI, ICMR and WHO.

***Table 3.*** *Standards for drinking water quality*

| S. No. | Parameters | BIS: 1999 | ICMR: 1975 | WHO: 2003 |
|---|---|---|---|---|
| 1. | pH | 6.5–8.5 | 7.0–8.5 | 6.5–9.5 |
| 2. | EC (μseimens/cm) | – | – | 1400 |
| 3. | TDS | 2000 | 500 | 600 |
| 4. | $Na^+$ | – | – | – |
| 5. | $K^+$ | – | – | – |
| 6. | $Ca^{2+}$ | 200 | 200 | 100 |
| 7. | $Mg^{2+}$ | 100 | 200 | 150 |
| 8. | $Cl^-$ | 1000 | 200 | 250 |
| 9 | $CO_3^{2-}$ | – | – | – |
| 10 | $HCO_3^-$ | – | – | – |
| 11 | $SO_4^{2-}$ | 400 | 200 | 250 |
| 12 | $NO_3^-$ | 100 | 50 | 50 |
| 13 | TH | 600 | 600 | 500 |

Note: All values except pH and EC are expressed in mg/l.
TDS = Total Dissolved Solids
EC = Electrical Conductance
TH = Total Hardness

## *Chemical classification of ground water*

*Hill-Piper Diagram*

One method of comparing the results of chemical analyses of ground water is with a trilinear diagram [9] (Figure 1). This diagram consists of two lower triangles that show the percentage distribution, on the milliequivalent basis, of the major cations ($Mg^{++}$, $Ca^{++}$, and $Na^+$ plus $K^+$) and the major anions ($Cl^-$, $SO4^{2-}$and $CO_3^{2-}$plus $HCO_3^-$) and a diamond-shaped part above that summarizes the dominant cations and anions to indicate the final water type. This classification system shows the anion and cation facies in terms of major-ion percentages. The water types are designated according to the area in which they occur on the diagram segments.

The cation distribution indicates that the samples range in composition from sodium/potassium to predominantly mixed cation. There is a small percentage of the ground water that has a calcium cation classification. In the anion triangle, there is a tendency toward chloride/bicarbonate type water to mixed anion-type water.


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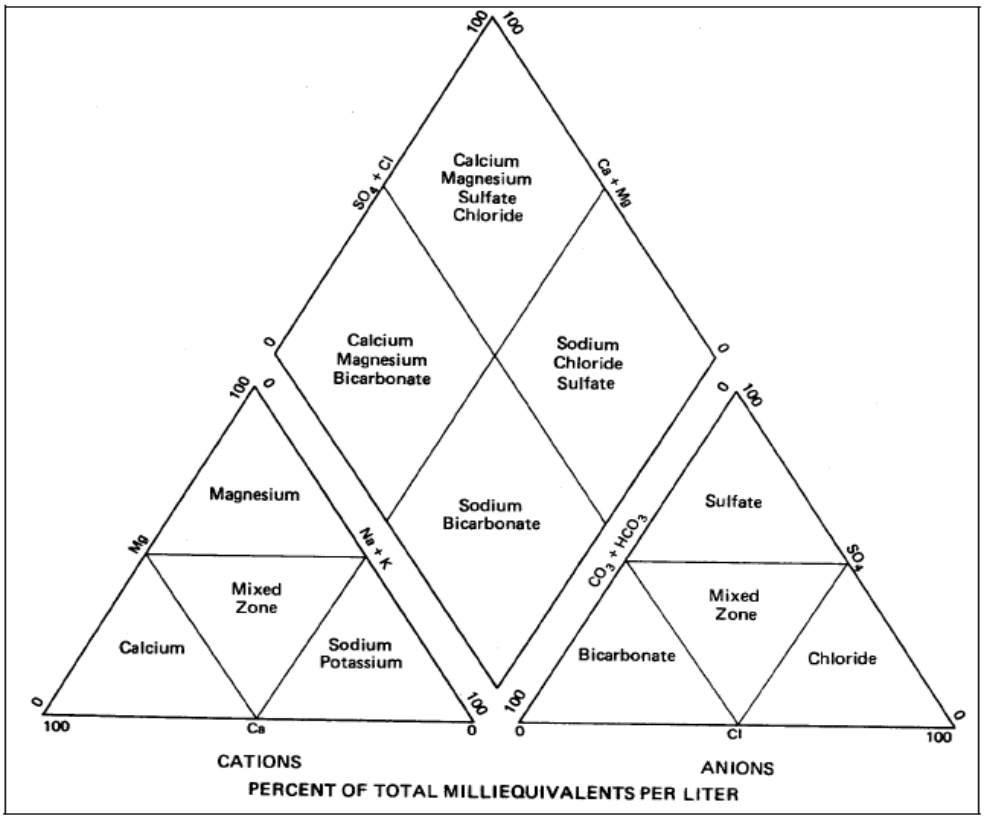


***Figure 1.*** *Trilinear diagram showing water type categories*

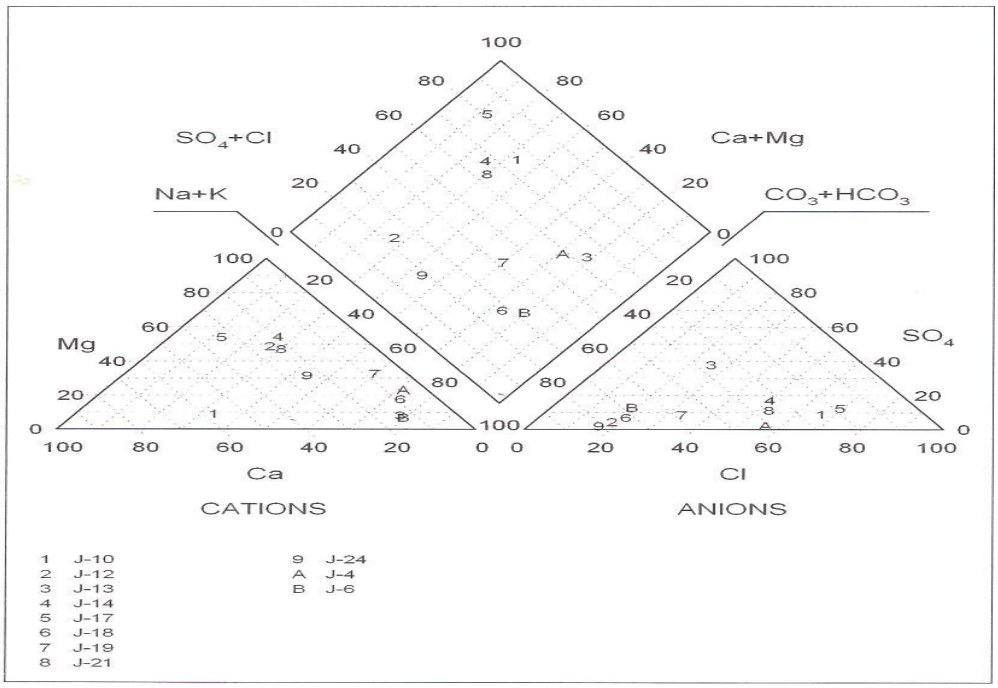


***Figure 2.*** *Piper diagram of ground water quality of Jaipur city*


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### *Groundwater quality for irrigation purposes*

The concentration and composition of dissolved constituents in a water determine its quality for irrigation use, several chemical constituents affect water suitability for irrigation from which the total concentration of the soluble salts and the relative proportion of sodium to calcium and magnesium. Moreover suitability of water for irrigation is depended on the effect of some mineral constituents in the water on both the soil and the plant (Wilcox, 1948 & 1955). The following are the important characteristic properties of ground water of determine its suitability of irrigation proposes.

#### *TDS*

Regarding to the TDS content the water is considered satisfactory when it contains lesser than 1000 mg/l, fair if it contains between 1000 to 2000 mg/l, and inferior when it salinity exceeds 2000mg/l. Accordingly, all sampling stations considered suitable for irrigation uses.

#### *EC*

The most influential water quality guideline on crop productivity is the salinity hazard as measured by electrical conductivity (EC). The primary effect of high EC water on crop productivity is the inability of the plant to compete with ions in the soil solution for water. The higher the EC, the less water is available to plants Nearly all irrigation waters that have been used successfully for a considerable time have conductivity less than 2250 microsiemens/cm.

#### *Sodium percentage (Na%)*

The sodium percentage was calculated by Todd's (1959) method:

$$Na\ \% = (Na+K)\ 100 / (Ca+Mg+Na+K) \quad (epm)$$

The sodium percentage in the study area ranges between 1.07 to 6.74 %. Na% values reflected that the water was under the category of 'good' (20-40 Na %), 'permissible' (40-60 Na%) and 'doubtful' (60-80 Na%) class according to Wilcox (1955).

### ***Sodium Absorption Ratio (SAR)***

SAR is an important parameter for determination of Suitability of irrigation water. The sodium hazard is typically expressed as the sodium adsorption ratio (SAR). This index quantifies the proportion of sodium ($Na^{+}$) to calcium ($Ca^{++}$) and magnesium ($Mg^{++}$) ions in a sample. Sodium hazard of irrigation water can be well understood by knowing SAR. Sodium adsorption ratio varied from 0.80 to 6.73 (Table 2). Todd (1980) classified irrigation water with SAR values less than 10 as 'excellent' and the water is evaluated suitable for any crop Lower the ionic strength of Sodium, greater the sodium hazard; and conversely, if Calcium and magnesium predominant, the hazard is low [16]. The sodium adsorption ratio (SAR) values of each water sample were calculated by using Richard (1954) equation:


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$$SAR = (Na^{+} \text{ meq/l}) / \sqrt{[(Ca^{2+} \text{ meq/l}) + (Mg^{2+} \text{ meq/l}) /2}$$

There is a significant relationship between SAR values of irrigation water and the extent to which sodium is adsorbed by the soil. If the water used for irrigation is high in Sodium and low in Calcium, the cation-exchange complex may become saturated with Sodium. This can destroy the soil structure owing to dispersion of clay particles .The calculated value of SAR in the study area ranges between 1.07 to 6.74. Data is plotted on the US salinity diagram (Figure No. 3), in which EC is taken as salinity hazard and SAR is taken as alkalinity hazard. The ground water samples (J-4, J-6, J-19) fall in the $C_3S_2$ quality with high salinity hazard and medium sodium hazard. Samples (J-10, J-17, J-21) lie in $C_4S_1$-very high salinity hazard and low sodium hazard. The ground water samples (J-5, J-14,) fall in the $C_3S_1$ quality with high salinity hazard and low sodium hazard. Samples (J-13, J-24, J-12) lie in $C_2S_1$-medium salinity hazard and low sodium hazard.

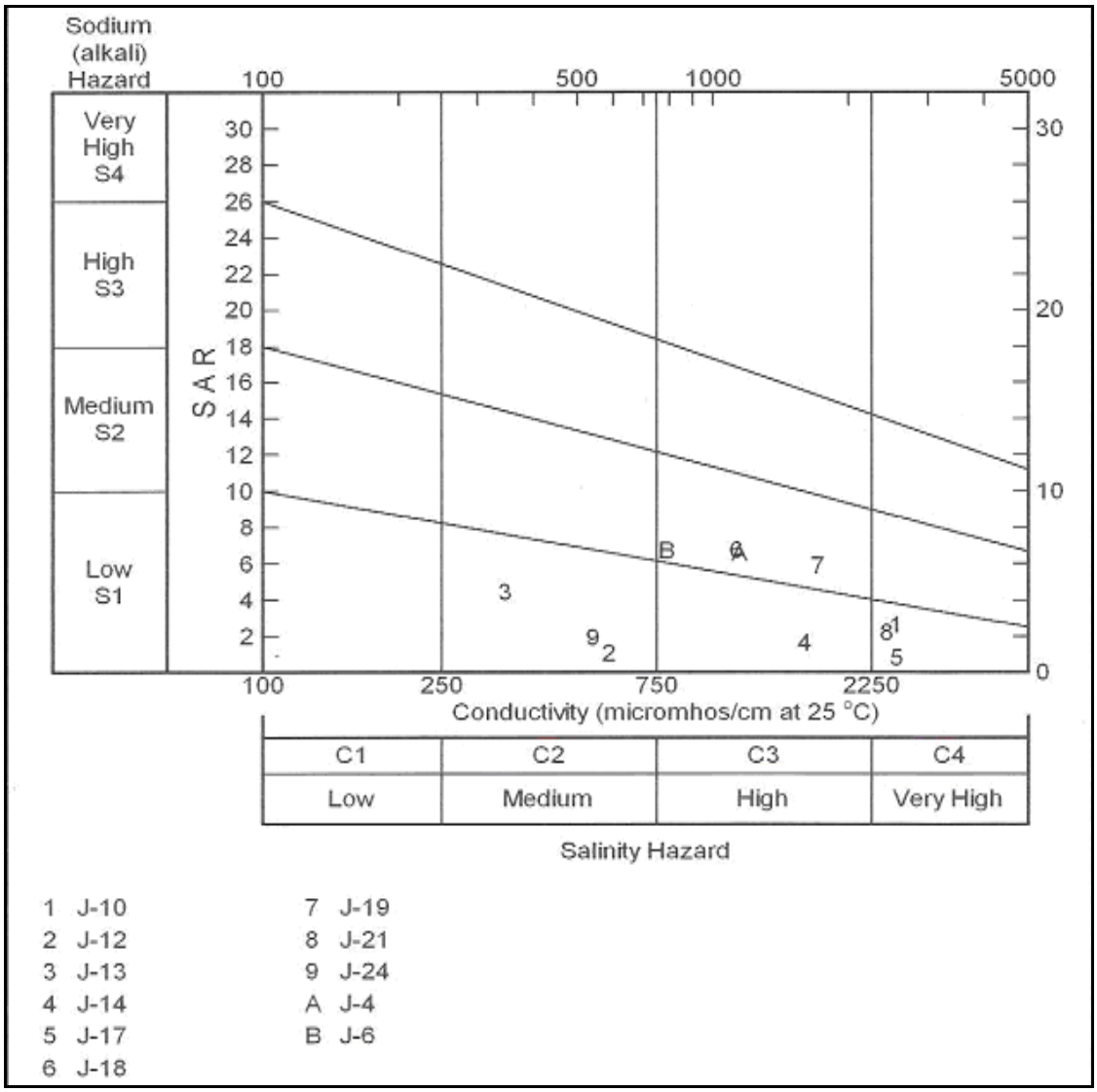


***Figure 3.*** *Water classification according to EC and SAR values*


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## Conclusion

It is observed that about 36% of ground water exceeds the permissible limit of EC, TDS and TH prescribed by (WHO: 2003). Almost all the parameters like pH, $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $SO_4^{2-}$, $CO_3^-$, $HCO_3^-$ and $Cl^-$ are within the permissible limits prescribed by prescribed by ISI, ICMR, WHO. On the other hand all the sampling station considered suitable for irrigation uses according to TDS, EC and SAR value. According to the quality classification of irrigation water proposed by Wilcox (1955), 45 % of the water falls in the doubtful category.

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# VEGETATION ECOLOGY OF THE SIMILIPAL BIOSPHERE RESERVE, ORISSA, INDIA

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**Abstract** Vegetation analysis of the forest ecosystem of Similipal Biosphere Reserve (SBR) was carried out at 10 sites to study changes in structure and composition in plant community distributed in the core (undisturbed) and buffer (disturbed) areas of the reserve. The study reveals a higher number of herbs and shrubs and a lower number of trees in the buffer area indicating greater anthropogenic disturbance. Total tree basal area varied from 48.7 to 78.61 $m^2 ha^{-1}$ in the buffer area and 81.4 to 104.9 $m^2ha^{-1}$ in the core area. The density of saplings and seedlings was nearly equal both at the disturbed and undisturbed sites. However, the rate of conversion of saplings to trees was greater at undisturbed sites. The lower rate of conversion at disturbed sites is due to the removal of seedlings of most of the tree species. The high herb diversity (2.14 – 3.50) and low tree diversity (2.14 – 2.98) in buffer area is a result of Environmental openings providing greater opportunity for the recruitment of herbs and shrubs. The presence of only a few individuals of major tree species in larger diameter classes and more in young diameter classes in buffer areas indicate that the plant community was subjected to disturbance and are in the regenerating stage. Greater fluctuation in the species / genus ratio in the herbaceous species at sites of the buffer area in comparison to core area has led to variation in developmental status of plant communities among the core and buffer areas of the reserve. However, the presence of the seedlings of dominant tree species in the buffer area may help in the restoration of the plant communities in the long-run, provided protection means are strengthened and biotic stress reduced.
**Keywords:** *Disturbance index, diversity, regeneration, community, succession, India*

## Introduction

Similipal Forest Ecosystem was brought into the Man and Biosphere Programme of UNESCO by declaring it as a Biosphere Reserve by the Government of India in 1994. Understanding the disturbance regimes of a forest landscape and its management under natural and semi natural conditions is essential for conservation of biological diversity (Spies and Turner, 1999). Disturbance is an extraordinary event and human generated deviation from the normal successional development of equilibrium communities (Oliver and Larson, 1990). Biodiversity is the totality of genes, species and ecosystem in a region. Apart from natural disturbances, human induced impacts have caused a significant loss to biodiversity especially in underdeveloped tropical regions. These regions have a greater diversity of plants and animals in natural habitats. Conservation biologists warn that 25 percent of all species could become extinct during the next twenty to thirty years. The loss of species is accelerating due to fragmentation of natural habitats. In the Similipal Biosphere Reserve (SBR) chronic disturbances are caused by people for their subsistence need. People remove a fraction of the forest biomass in the form of grazing, lopping, surface burning and litter removal at regular time intervals


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(Raut and Behera, 1997; Parida, 1997). Illicit cutting and removal of timber has also now become a prevalent practice, which impacts the regeneration potential of sites. The problem with these forms of forest disturbances is that the plants often do not have time to recover adequately and thus, these impacts affect the ecosystem succession of the communities.

Champion and Seth (1968) and Saxena and Brahmam (1989) have described the species composition of SBR. Some productivity-related parameters of the keystone species of SBR have been studied by Bal (2002). A comprehensive study on the structural parameters i.e. Phytosociology of tree, shrub and herb species of SBR has been carried out by Mishra et al. (2003). The present study deals with changes in vegetation diversity, composition and regeneration pattern of tree species in SBR in relation to natural and anthropogenic disturbances.

## Materials and methods

The study area (SBR) is located between 21º28'- 22º 08' N latitude and 86º04' - 86º37' E longitude and with a range in elevation of 80 to 869 m. For a detailed study of plant diversity and other vegetational parameters, the area was divided into 10 randomly selected sites in East, West, North and South regions of the reserve.

The climate is influenced by a monsoon pattern of rainfall. The annual rainfall is 3450 mm; three-fourths of which occurs in the rainy season (mid June to September). The mean monthly maximum temperature ranges between 34.4ºC (May) and 37.4 ºC (June), and minimum between 7.2 ºC (December) and 11.1 ºC (January).

Vegetation analysis was conducted during 2000-2001 for all the three layers of the forest i.e. trees, shrubs and herbs. The species were identified with flora guides including Saxena and Brahmam, 1994-1996 (Haines, 1921-25). The tree layer was analyzed by sampling 20 quadrats of 10 x 10 m size at each site. The size and number of samples were determined using the method of Misra (1968) and Kershaw (1973). The abundance, density and frequency were calculated for the species. IVI was determined as the sum of the relative frequency, relative density and relative dominance for tree layer only. The distribution pattern of different species was studied using the ratio of abundance to frequency (Whitford, 1949) and the degree of disturbance following Pandey and Shukla (1999). The physical condition of each individual tree present inside the 10 x 10 m quadrat was noted under normal and damaged categories. The trees involved under normal categories were the healthy individuals. The damaged category included the individuals that were partly broken at the top, partly dry or had recently fallen. The Individuals that were standing dead, cut stumps and completely dry were also recorded. Because such plants create problem for identification, they were not identified to species. Trees were > 31cm cbh (circumference at breast height), saplings were 10-31 cm cbh and seedlings were <10cm cbh (Knight, 1975). The shrub and herbs layers were analyzed by randomly placing 20 quadrats of 5 x 5m size and 1 x 1m size respectively at each site during the post monsoon season. The diversity index at each site was computed by using Shannon- Wiener information function (Shannon-Wiener, 1963) and concentration of dominance by Simpson's index (Simpson, 1949), evenness and richness index following Pielou (1975) and Margalef (1958), respectively. The species/genus ratio (S/G) was computed following Ricklefs and Miller (2000) to study the degree of succession.


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## Results

### *Inventory of vascular plants*

A total of 203 species were recorded from the study area, out of which 98 were tree species, 29 shrub and 76 herb species. Thus only approximately 19% of the estimated flora of Similipal (Saxena and Brahmam, 1989) was covered in the study. There was a considerable difference in the species number among the study sites with site-2, being the richest having 64 species and the sites 6 and 9 having the lowest number of species (42). The other study sites showed an intermediate range (Table-1).

***Table 1.*** *Characteristics of study sites in Similipal Biodiversity Reserve in Orissa, India*

| Site | Aspect | Elevation | Normal trees | Damaged trees | Total (Normal + Damaged) | D.I. (%) | Level of interference |
|---|---|---|---|---|---|---|---|
| Podadiha ($S_1$) | South-East | 80 | 109 | 84 | 193 | 43.52 | HB |
| Bangirposi($S_2$) | East | 226 | 107 | 81 | 188 | 43.68 | HB |
| Handipuhan($S_3$) | North | 280 | 111 | 96 | 207 | 46.38 | HB |
| Ghodabindha($S_4$) | West | 557 | 105 | 82 | 187 | 43.85 | HB |
| Kendumundi($S_5$) | West | 400 | 157 | 57 | 214 | 26.63 | MB |
| Kalika Prasad($S_6$) | West | 468 | 138 | 44 | 182 | 24.17 | MB |
| Joranda ($S_7$) | North | 681 | 170 | 25 | 195 | 12.80 | NB |
| Chahala ($S_8$) | North | 774 | 168 | 29 | 197 | 14.72 | NB |
| UBK ($S_9$)* | South | 824 | 186 | 36 | 222 | 16.22 | NB |
| Jenabil ($S_{10}$) | South | 869 | 175 | 31 | 206 | 15.05 | NB |

D.I. (Disturbance Index) = Percentage of damaged individuals of the total number of woody individuals per 2000 $m^2$ area.*- UBK – Upper Barakanda.
HB- High biotic interference; MB- Moderate biotic interference; NB- No biotic interference.

Middle elevation sites had a lower number of species than lower and higher elevation sites. Disturbed sites contained more species than undisturbed and moderately disturbed sites. Disturbances occurred either in the form of recurring soil erosion (natural) or anthropogenic disturbances such as grazing, lopping, surface burning and illegal cutting of trees (Table 1 and 2).

***Table 2.*** *Spatial distribution of species*

| Site | Tree | Shrub | Herb | Total |
|---|---|---|---|---|
| S – 1 | 29 | 8 | 16 | 53 |
| S - 2 | 31 | 12 | 21 | 64 |
| S -3 | 20 | 10 | 13 | 43 |
| S - 4 | 27 | 10 | 13 | 50 |
| S - 5 | 36 | 7 | 8 | 51 |
| S - 6 | 23 | 7 | 12 | 42 |
| S - 7 | 31 | 8 | 10 | 49 |
| S - 8 | 36 | 8 | 10 | 54 |
| S - 9 | 19 | 9 | 14 | 42 |
| S-10 | 31 | 9 | 12 | 52 |


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*Structure of the vegetation*

Importance value index (IVI) of tree species indicated that *Shorea robusta* Gaertn.f., was the dominant species at all the study sites in the tree and sapling layers of the reserve followed by *Dillenia pentagyna*, *Terminalia alata*, *Anogeissus latifolia*, *Schleichera oleosa*, *Syzygium cumini*. The IVI of the dominant tree species was less (111.51 to 151.37) at the disturbed sites and more (180.64 to 235.63) at the undisturbed sites. While intermediate range of IVI (158.58- 207.73) of dominant tree species was found in the moderately disturbed study sites. No such clear-cut difference was observed in the IVI of dominant tree species due to altitude or aspect (Table 3).

The tree density across the sites ranged from 650 to 970 individuals ha $^{-1}$ and the basal area from 48.71 to 104.92m$^2$ ha $^{-1}$. The density and basal area of undisturbed sites was higher than the disturbed sites. However, the difference in density and basal area of the shrub and sapling layer among the disturbed and undisturbed sites of the reserve was not distinct. Diversity of herb species was much higher at the undisturbed sites. (Table 4).

*Distribution pattern*

The distribution pattern of trees, shrubs, herbs, saplings and seedlings for all study sites is shown in Table 5. Odum (1971) stated that under natural conditions, a clumped distribution of plants is normal. A higher percentage of random and regular distribution reflects the greater magnitude of disturbance` such as grazing and lopping in natural forest stands. All the vegetational layers showed generally clumped type of distribution in the present study.

***Table 3.*** *Importance Value Index (IVI) of most dominant tree species of Similipal Biosphere Reserve*

| **Name of the plant species** | **S-1** | **S-2** | **S-3** | **S-4** | **S-5** | **S-6** | **S-7** | **S-8** | **S-9** | **S-10** |
|---|---|---|---|---|---|---|---|---|---|---|
| *Adina cordifolia* (Roxb.) Hook. F. ex. Brandis | 7.85 | 5.38 | 18.03 | 2.17 | 10.14 | 8.32 | 7.05 | 3.70 | - | 3.73 |
| *Anogeissus latifolia* (Roxb. Ex DC) Wall ex. Bedd. | 14.22 | 14.4 | 13.60 | 25.22 | 34.06 | 11.64 | 25.0 | 9.65 | - | - |
| *Bombax ceiba* L. | - | 1.93 | - | - | 3.32 | - | 4.03 | 6.35 | 5.14 | 27.43 |
| *Dillenia pentagyna* Roxb. | 14.15 | 8.80 | 5.58 | - | 6.9 | 8.11 | 15.73 | 57.43 | 20.58 | 54.13 |
| *Shorea robusta* Gaertn. f. | 37.12 | 41.77 | 52.69 | 84.76 | 73.43 | 149.92 | 107.49 | 51.27 | 130.15 | 53.75 |
| *Syzygium cumini* (L.) Skeels | - | 7.16 | 8.99 | - | 2.69 | 14.71 | 1.97 | 23.95 | 12.12 | 14.59 |
| *Syzygium cerasoides* (Roxb.) Raizada | 6.53 | - | - | - | 4.04 | 9.34 | 11.76 | 4.02 | 14.05 | 7.71 |
| *Schleichera oleosa* (Lour.) Oken | 15.1 | 8.29 | 5.25 | 11.15 | 6.49 | - | 5.19 | 13.19 | 3.57 | 13.59 |
| *Terminalia alata* Heyne ex Roth. | 29.23 | 22.13 | 13.38 | 21.49 | 13.27 | 3.16 | 25.09 | 11.08 | 46.57 | 15.52 |
| *Terminalia chebula* Retz. | 3.03 | 1.65 | 2.69 | 5.58 | 4.24 | 2.53 | 2.57 | - | 3.45 | 5.16 |


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***Table 4.*** *Importance Value Index (IVI) of most dominant tree species of Similipal Biosphere Reserve Density (individuals ha –1) and Basal area (m 2 ha-1) of different vegetational layers of Similipal Biosphere Reserve.*

| Site | Density | | | | Basal area | | |
|---|---|---|---|---|---|---|---|
| | Tree | Saplings | Shrubs | Seedlings | Herbs | Tree | Saplings |
| S - 1 | 680 | 1500 | 640 | 12.40 | 42.4 | 52.36 | 7.07 |
| S - 2 | 675 | 1160 | 600 | 9.00 | 37.5 | 59.54 | 6.84 |
| S -3 | 715 | 1220 | 580 | 18.05 | 33.0 | 48.71 | 6.57 |
| S - 4 | 650 | 920 | 580 | 8.00 | 21.0 | 49.13 | 6.61 |
| S - 5 | 845 | 1320 | 400 | 9.00 | 6.0 | 68.99 | 6.11 |
| S - 6 | 750 | 2040 | 460 | 16.00 | 5.0 | 78.61 | 5.68 |
| S - 7 | 810 | 1200 | 280 | 20.00 | 3.0 | 81.35 | 4.40 |
| S - 8 | 875 | 1380 | 200 | 9.00 | 2.0 | 88.59 | 5.18 |
| S - 9 | 970 | 1620 | 180 | 8.70 | 2.5 | 84.86 | 7.21 |
| S -10 | 895 | 1720 | 260 | 17.60 | 2.0 | 104.92 | 6.67 |

The tree layer exhibited less clumped distribution at the disturbed sites than the undisturbed and moderately disturbed study sites. The distribution pattern of other vegetational layers did not show any distinct difference among the study sites. The trees at Podadiha, Bangirposi, Handipuhan and Ghodabindha are faced with more biotic disturbance; Kendumundi and Kalika Prasad with moderate biotic disturbance; and Joranda, Chahala, UBK and Jenabil with no biotic disturbance.

***Table 5.*** *Distribution pattern (%) of plant layers.*

| Site | Stratum | Regular | Random | Contiguous |
|---|---|---|---|---|
| HB | Tree | 17-23 | 33-45 | 35-48 |
| | Sapling | 4-10 | 24-46 | 50-67 |
| | Shrub | 2-5 | 28-40 | 57-72 |
| | Herb | 0-5 | 5-20 | 75-95 |
| | Seedling | 0-4 | 3-14 | 82-87 |
| MB | Tree | 9-14 | 33-35 | 53-57 |
| | Sapling | 5-8 | 12-25 | 69-81 |
| | Shrub | 5-6 | 22-42 | 53-72 |
| | Herb | 0-5 | 8-20 | 75-92 |
| | Seedling | 0-0 | 3-7 | 93-97 |
| NB | Tree | 0-5 | 19.44-32.25 | 67.74-77.78 |
| | Sapling | 0-5 | 7.69-25 | 70-88.46 |
| | Shrub | 0-8 | 30-40 | 55-61 |
| | Herb | 0-0 | 3-24 | 76-97 |
| | Seedling | 0-0 | 6.89-15.38 | 84.61-93.10 |

Species diversity, concentration of dominance and some mathematical indices of different vegetational layers of the study sites are given in Table 6. Measurement of biodiversity of specific area (local scale) on the basis of species richness does not provide a complete understanding about the individuals of the species in an ecosystem as it suffers from the lack of evenness or equitability.


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***Table 6.*** *Species diversity (SD), Concentration of dominance (CD) species richness (SR) and species evenness (SE) of different forest strata*

| Site | Parameters | Vegetational Layer | | | | |
|---|---|---|---|---|---|---|
| | | **Tree** | **Sapling** | **Shrub** | **Seedling** | **Herb** |
| S - 1 | SD | 2.139 | 2.512 | 2.676 | 2.151 | 2.519 |
| | CD | 0.103 | 0.113 | 0.095 | 0.178 | 0.117 |
| | SR | 5.69 | 3.68 | 4.135 | 3.156 | 2.793 |
| | SE | 0.635 | 0.873 | 0.887 | 0.759 | 0.895 |
| S - 2 | SD | 2.98 | 2.135 | 2.114 | 1.746 | 3.496 |
| | CD | 0.072 | 0.166 | 0.176 | 0.318 | 0.092 |
| | SR | 6.116 | 2.875 | 3.301 | 2.962 | 2.537 |
| | SE | 0.868 | 0.832 | 0.851 | 0.629 | 1.209 |
| S -3 | SD | 2.426 | 2.033 | 2.067 | 2.905 | 2.446 |
| | CD | 0.131 | 0.192 | 0.161 | 0.140 | 0.128 |
| | SR | 3.828 | 2.676 | 2.532 | 2.732 | 1.815 |
| | SE | 0.809 | 0.818 | 0.817 | 1.072 | 0.798 |
| S - 4 | SD | 2.072 | 2.424 | 2.137 | 2.446 | 2.334 |
| | CD | 0.115 | 0.111 | 0.134 | 0.205 | 0.140 |
| | SR | 4.142 | 3.202 | 2.871 | 3.498 | 1.918 |
| | SE | 0.629 | 0.918 | 0.928 | 0.846 | 1.014 |
| S - 5 | SD | 2.887 | 2.465 | 1.785 | 2.615 | 2.138 |
| | CD | 0.093 | 0.113 | 0.191 | 0.128 | 0.136 |
| | SR | 6.823 | 4.043 | 2.004 | 2.962 | 1.677 |
| | SE | 0.806 | 0.853 | 0.917 | 0.822 | 0.980 |
| S - 6 | SD | 1.994 | 1.821 | 1.996 | 1.71 | 2.152 |
| | CD | 0.313 | 0.106 | 0.116 | 0.430 | 0.139 |
| | SR | 4.390 | 2.581 | 2.895 | 1.567 | 2.281 |
| | SE | 0.636 | 0.794 | 0.881 | 0.822 | 0.866 |
| S - 7 | SD | 2.896 | 1.956 | 1.955 | 2.105 | 2.043 |
| | CD | 0.193 | 0.216 | 0.158 | 0.257 | 0.188 |
| | SR | 5.897 | 2.676 | 2.377 | 3.673 | 1.901 |
| | SE | 0.843 | 0.787 | 0.940 | 0.743 | 0.797 |
| S - 8 | SD | 3.083 | 2.543 | 2.023 | 2.521 | 1.803 |
| | CD | 0.07 | 0.083 | 0.139 | 0.142 | 0.190 |
| | SR | 6.776 | 3.833 | 2.818 | 4.370 | 1.465 |
| | SE | 0.86 | 0.864 | 0.973 | 0.828 | 0.946 |
| S - 9 | SD | 1.798 | 2.379 | 2.082 | 3.251 | 2.048 |
| | CD | 0.316 | 0.136 | 0.148 | 0.139 | 0.179 |
| | SR | 3.417 | 3.729 | 2.525 | 4.116 | 1.837 |
| | SE | 0.611 | 0.839 | 0.982 | 1.308 | 0.723 |
| S-10 | SD | 3.107 | 2.763 | 2.043 | 1.913 | 2.084 |
| | CD | 0.069 | 0.093 | 0.136 | 0.207 | 0.199 |
| | SR | 5.803 | 3.723 | 2.509 | 2.245 | 2.071 |
| | SE | 0.905 | 0.786 | 0.841 | 0.798 | 0.839 |

It was observed that the richness index ranged from 3.48 to 6.12 (tree layer), 1.47 to 2.79 (herb layer), 2.00 to 4.14 (shrub layer), 2.58 to 4.04 (sapling layer) and 1.57 to 4.37 (seedling layer) and equitability showed little variation across the sites which ranged from 0.61 to 0.91 (tree layer), 0.72 to 1.21 (herb layer), 0.82 to 0.98 (shrub layer), 0.79 to 0.92 (sapling layer) and 0.74 to 1.31 (seedling layer). Shannon Wiener's index of diversity is one of the popular measures of species diversity. It ranged from 1.80 to 3.11, 1.80 to 3.50 and 1.79 to 2.68 for tree, herb and shrub layers, respectively, across all sites. The species diversity of the tree layer generally was highest at the undisturbed


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sites (except UBK) and lowest at the disturbed sites (except Bangirposi), but herb and shrub layers exhibited an opposite trend.

We considered seven cbh classes including seedlings (< 10 cm), saplings (10-31cm), bole (32-66 cm), post bole (67-101 cm), small (102-136cm), large (137-171cm) and over mature (>171 cm) following Singh et al. (1986). Density of woody tree species in different cbh classes in undisturbed and moderately disturbed study sites showed a continuous pattern in the order of seedling >sapling >bole >Post bole >small >large >over mature tress. However, on disturbed study sites, the order was seedling >sapling >bole < post bole < small >large > over mature in case of *Shorea robusta* and seedling > sapling > bole < post bole >small > large = over mature incase of *Dillenia pentagyna* and *Terminalia alata*.

*Stand Structure and Size Class Distribution:*

Cbh distribution of tree species among sites is largely controlled by the density of overstorey species and the pattern of regeneration can be described by the size distribution as reported by Singh et al. (1986). Density of individuals of the most dominant tree species such as *Shorea robusta*, *Dillenia pentagyna* and *Terminalia alata* in different cbh classes of disturbed, undisturbed and moderately disturbed study sites is presented in Table 7.

***Table 7.*** *Distribution (Number of individuals ha –1 ) of most dominant tree species in the disturbed, moderately disturbed and undisturbed study sites of Similipal Biosphere Reserve*

| **Girth class (cm)** | **Dominant tree species** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ***Shorea robusta*** | | | ***Terminalia alata*** | | | ***Dillenia pentagyna*** | | |
| | **DS** | **MDS** | **UDS** | **DS** | **MDS** | **UDS** | **DS** | **MDS** | **UDS** |
| < 10 (seedlings) | 16670 | 15600 | 15250 | 2334 | 2500 | 2400 | 1980 | 2050 | 2250 |
| 10-31 (Saplings) | 387 | 370 | 385 | 135 | 160 | 140 | 58 | 72 | 75 |
| 32-66 (Bole) | 24 | 90 | 117 | 20 | 30 | 70 | 15 | 14 | 15 |
| 67-101 (Post Bole) | 58 | 64 | 92 | 45 | 25 | 60 | 28 | 11 | 13 |
| 102-136 (Small) | 84 | 34 | 39 | 20 | 10 | 35 | 10 | 08 | 11 |
| 137-171 (Large) | 28 | 30 | 34 | 05 | 05 | 25 | 0 | 06 | 08 |
| > 171 (Over mature) | 10 | 18 | 24 | 05 | 05 | 10 | 0 | 05 | 06 |

DS: Disturbed sites, MDS Moderately disturbed sites, UDS: Undisturbed sites


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## Discussion

The forest ecosystems of Similipal Biosphere Reserve are experiencing disturbances of various magnitudes. Practices for removal of forest biomass in the form of grazing, lopping, surface burning and litter removal at a given time is a continuous disturbance affecting the stability of the ecosystem and retarding the successional processes in the area. These areas can be clearly demarcated on the basis of different phytosociological parameters of the vegetation.

Tree density in the different tropical forest has been recorded in the range of 550-1800 individuals ha $^{-1}$ (Visalakshi, 1995) and 3700 individuals ha $^{-1}$ in lowland neotropical dry forests (Gentry, 1995). In the present study the tree density ranged from 650 to 970 individuals ha $^{-1}$, which is comparable to tropical forests. Tree and sapling density were higher in the core area (undisturbed sites) compared to buffer area (disturbed sites). Higher anthropogenic disturbances in the buffer area has also led to the elimination of seedlings of most of the species (table-4).

The total basal area across the sites ranged from 48.7 to 104.9m$^2$ ha $^{-1}$ (Table - 4). The differences in basal area of tree layer among study sites may be due to difference in altitude, species composition, age of trees, degree of disturbance and successional stages of the stands. The value obtained for basal area in the present study is comparable to the Indian tropical forests (Visalakshi, 1995).

Importance value index of *Shorea robusta* in the present study area located at 80-869m altitude ranged from 37.12 to 149.92 which is comparable with the IVI of 127 (300-900m) and 181 (up to 1200 m) reported for *Shorea robusta* in mixed forests of Central Himalaya (Tewari & Singh, 1987). The change in IVI of *Shorea robusta* among the study sites is due to the change in species composition, disturbance and altitude (Procter et al., 1988). Suderpandian and Swamy (2000) reported that the Dipterocarpaceous members could not be found at higher altitudes (1150 m).

The species richness of trees ranged from 19 to 36. Other studies elsewhere have reported a similar range of species richness (Brockway, 1998; Tripathi, 2001). In the present study the species richness is positively correlated with species diversity ($r= 0.82$ and $0.82$ at $p< 0.01$ for trees and saplings, $r = 0.92$ for shrubs at $p< 0.001$ and $r = 0.66$ for herbs at $p<0.05$). While species diversity (Shannon wiener diversity index) and dominance (Simpson's index) are inversely related to each other ($r= -0.70$, $-0.65$ and $-0.72$ at $p<0.05$ for trees, saplings and shrubs, respectively and, $r = -0.82$ and $0.81$ at $p< 0.01$ for seedlings and herbs, respectively). The tree density and shrub diversity had a weak negative relation with each other and the relationship is not being significant. Tree density and herb diversity ($r = -0.65$ $p < 0.05$) were highly negatively correlated, establishing that the open canopy provides opportunity for the recruitment of shrubs and herbs.

The diversity index is generally higher for tropical forests and the reported range is 5.1 and 5.4 for young and old stands, respectively (Knight, 1975). Many researchers have reported the diversity value for Indian forests in the range of 0.8 to 4.1 (Parthasarthy et al., 1992; Visalakshi, 1995). Thus, the diversity values of tree species obtained in the present study is well within the reported range of Indian tropical forests. However, these values are lower than the other tropical forests (e.g. Knight, 1975) which may perhaps be due to climatic differences and high degree of natural disturbances, which are critical factors in governing the tropical forest species diversity (Foster, 1990). The lower diversity values in Indian forests may also be due to


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anthropogenic disturbances such as burning, grazing and wood collection (Jayasingam and Vivekanantharaja, 1994).

The population structure and size class distribution have generally been used by many researchers for understanding regeneration and magnitude of disturbances and future stability of tree species population in forest communities (Upreti, 1982). From the present study, the overall pattern of distribution of the trees in different chb classes reveals dominance of mature trees at the undisturbed sites and seedlings and saplings at the disturbed sites. More number of individuals of dominant tree species, such as S*horea robusta*, *Dillenia pentagyna* and *Terminalia alata* in higher cbh classes were observed in the core area. This may indicate that buffer area has had more anthropogenic disturbance. A similar pattern has also been reported by Khan et al. (1987) among the disturbed and protected subtropical forest sites of northeast India.

The data on species/genus ratio helps to compare the rate of species development because high ratio indicate recent diversification (Fig.1). Tropical areas have low species/genus ratio, indicating that the tropical species have emerged over a long period of time (Ricklefs and Miller, 2000). In the present study, all the study sites show lower S/G ratio in the tree and shrub layer and higher S/G ratio in the herb layer, thus supporting the findings of Ricklefs and Miller (2000). Further studies on functional parameters of the ecosystem shall provide more information to establish linkages and interactions between edaphic factors, productivity, nutrient input and decomposition and structural characteristics of the vegetation of Similipal Biosphere Reserve which will help management authorities to take measures for restoration.

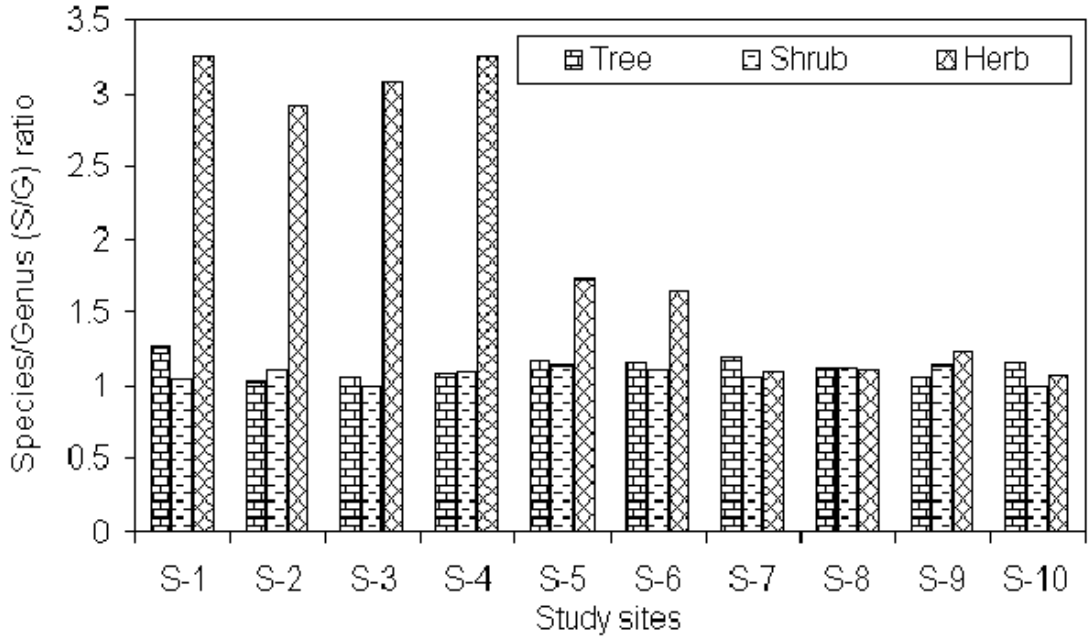


***Figure 1.*** *Species/Genus ratio of different vegetational layers of Similpal Biosphere Reserve*


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# SCREENING OF NITROGEN FIXERS FROM RHIZOSPHERIC BACTERIAL ISOLATES ASSOCIATED WITH IMPORTANT DESERT PLANTS

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**Abstract.** Free living nitrogen fixers represent a range of microorganisms including bacteria living on plant residues (saprophytes), bacteria which live entirely within plants (endophytes) and bacteria living in close association with the plant roots (rhizobacteria). We have isolated a number of rhizosphere associated bacteria from two of the hot arid zone plants and explored these in terms of nitrogen fixing ability both in solid and liquid culture conditions. The maximum coloring zone was developed in T-1 (22 mm) whereas minimum was in T-3 (4 mm) in case of the C. polygonoides associated bacterial community. The coloring zone was found maximum in TS-2 (27 mm) and minimum in TS-1 (11 mm), in case of isolates associated with rhizosphere of L. sindicus. The highest and lowest acetylene reduction activity (ARA) was detected in TS-13 (8303 n moles / 24 h) and T-10 (1658 n moles / 24 h), respectively.
**Keywords** *Hot arid region, rhizospheric bacteria, nitrogen fixation, ARA, malate media.*

## Introduction

The rhizosphere or the zone of influence around plant roots harbors a multitude of microorganisms that are affected by both abiotic and biotic stresses. Among these are the dominant rhizobacteria that prefer living in close vicinity to the root or on its surface and play a crucial role in soil health and plant growth [6]. These groups of bacteria are important as they are involved in various soil biochemical processes viz. fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores that solubilize and sequester iron or production of plant growth regulators [9].

Nitrogen (N) is the key plant nutrient required for plant growth. The elemental N is abundant in the earth's atmosphere [12] however; most of the tropical soils are deficient in available N. In ecosystems with low inputs and without any fertilization or soil amendments by humans, the nutrients available to plants come either from atmospheric inputs [3] or from biological fixation [15]. Biological nitrogen fixation (BNF) is one way of converting elemental nitrogen into plant usable form. A number of microbes are involved in the process of BNF, which contains nitrogenase enzyme responsible for fixing atmospheric dinitrogen into soil, thus improving the soil fertility. Therefore, it is an excellent, economically and environmentally sound source to substitute fertilizer N [14]. The BNF is estimated to contribute 180 x $10^6$ metric tons/year globally and about 80% comes from symbiotic associations. Besides, symbiotic nitrogen fixation, the non symbiotic nitrogen fixation is also known to be of great agronomic significance [19].

The arid zone of India covers an area of 38.7 m ha, out of which 31.7 m ha comes under hot arid zone. The soils in this zone are coarse textured and covered with sand


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dunes. Low available water capacity, vulnerability to wind erosion and low fertility are major constraints along with high salinity, calcareousness and gypsiferous nature [4]. The temperature rises up to 50ºC with average rainfall less than 200 mm causing the conditions more severe for most of the life forms besides poor nutritional status of the soils [1]. The plant and microbial diversity in these areas are limited, which proves their ecological importance. Sand dunes from Thar are reported to have relatively smaller population of microorganisms ($1.5 \times 10^2 - 5 \times 10^4$ per g soil) [7, 20] because of the extreme variations of environmental factors. The enhancement of soil fertility in these areas thus requires a careful management of natural resources for sustainable plant production. This involves the concept of using, improving and restoring the productive capacity and life support processes of soil [16]. Bio-inoculants production is one approach in these areas.

Two plant species (viz. *Calligonum polygonoides and Lasiurus sindicus*) were selected on basis of their ecological importance and well adaptability in these hot arid areas in order to study the rhizosphere associated bacterial diversity. During the investigation of plant growth promoting bacterial inoculants (development of bio-inoculants) for improving plant production of the selected plant species we found some phosphate solubilizing bacteria [5] and a number of nitrogen fixing bacteria. The objective of the present investigation was to screen the plant rhizosphere associated nitrogen fixing bacterial isolates and to test their efficiency in nitrogen fixation under liquid culture conditions.

## Materials and methods

### *Study site and soil sampling*

In the western desert region of Rajasthan *C. polygonoides* (Phog) and *L. sindicus* (Sewan grass) plants are known for their involvement in the ecological maintenance by helping in the soil conservation and dune stabilization besides some economical values e. g. wood, fuel, fodder etc. Therefore, these two plants have achieved a prominence due to their adaptability in desert climatic conditions in the Western sandy plain sub-region, which is one of the agroecological zone of North Western hot arid regions of India. Soil samples were collected from the rhizosphere of these two plants covering three different zones of this sub-region. These zones have been characterized by dune complex physiography and poor surface and ground water resources [4].

### *Isolation and characterization of bacterial cultures*

Different bacterial morphotypes were isolated from 1 g rhizosphere soil sample on King's B media [8] as described by Gothwal et al. 2006 [5] at both 37ºC and 50ºC respectively and characterized the important biochemical properties using the standard protocol [17].

### *Screening of nitrogen fixing bacteria*

Nitrogen free Malate media [13], containing bromothymol blue (BTB) as an indicator, was used for preliminary screening and incubated at 37ºC and 50ºC up to 24 h. The blue coloured zone producing isolates were marked as nitrogen fixers in the solid culture conditions. The colouring zone was calculated by deducting the colony diameter from the colouring zone diameter. To determine whether these isolates were truly


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nitrogen fixers, they were further tested for their acetylene reduction activity (ARA) assay in liquid culture.

### *Quantification of nitrogen fixation*

To quantify the nitrogen fixation the different morphotypes were inoculated in nitrogen free Malate broth in 250 ml Erlenmeyer flasks and incubated in an orbital shaker at 180 rpm at 37ºC and 50ºC temperatures for 24 – 48 h. Five ml of well grown culture was then transferred to 45 ml fresh sterile media and incubated for 72 h at the same conditions. Then 20 ml of this enriched culture was transferred into 30 ml capacity glass screw cap autoclaved vials and cap was replaced with subaseal. With the help of a 5 ml sterile syringe 3 ml air was replaced with same amount of acetylene gas and again incubated in shaker up to 24 h. Nitrogen fixation of the isolates was quantified by measuring the ethylene production by acetylene reduction assay. Analysis of the produced ethylene was accomplished by gas chromatography (Nucon-5765, AIMIL Instruments) using Porapak N column (200 x 0.2 cm) equipped with a flame ionization detector (FID) in isocratic condition. The operating conditions were as follows: temperature of oven 80ºC; the injector temperature was kept 200ºC; and the detector temperature was maintained at 200ºC. The flow of $N_2$ gas was maintained 42 ml/min, $H_2$ at a flow rate of 64 ml/min and air at a flow rate of 42 ml/min respectively. One ml of sample gas was injected and the area of ethylene was calculated against the standard ethylene (Spancan Calibration Gas, Spantach Products, England). The amount of ethylene produced is represented in the terms of nano moles of ethylene formed in 20 ml of broth at 30ºC after 24 h of incubation. All the experiments were conducted in triplicate along with a control (without inoculation). The mean values of each experiment were calculated and reported.

## Results and discussion

More than fifty different colonies were isolated during the study of rhizosphere associated bacterial species in case of *C. polygonoides* and *L. sindicus*. In continuation of the work towards development of growth promoting inoculants we found that there is a large number of free nitrogen fixers associated with the rhizosphere of these two plant species.

### *Screening of nitrogen fixers*

The nitrogen fixing bacteria were preliminary screened on nitrogen free Malate agar media containing BTB as an indicator. Different isolates associated with rhizosphere of *C. polygonoides* and *L. sindicus* were cultivated at different temperatures (37ºC and 50ºC) to observe the growth characteristics. Figure 1 depicts the highest and lowest zones of coloration developed by both of the plant associated rhizotypes. Isolates T-1, 2, 3, 4, 5, 6, 8, 10, T-22 and TS-1, 2, 3, 4, 5, 11, 12, 16, 17, 18, TS-20 were found to grow maximally at 37ºC. Similarly, T-11, 12, 13, 14, 15, 17, 20, 21, T-23 and TS-6, 7, 8, 9, 13, 14, 15, TS-19 were found to grow at 50ºC. The maximum zone of coloration in case of the isolates associated with rhizosphere of Phog was observed in T-1 (22 mm) whereas; lowest value was detected in T-3 (4 mm), as shown in Figure 2. Some of the isolates which were grown on higher temperature i.e. 50ºC, also showed the zone of


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coloration on nitrogen free malate media containing BTB. In such a case, the maximum zone of coloration was detected in T-12 (17 mm) and minimum in T-15 (7 mm).

In case of the isolates associated with rhizosphere of Sewan grass, the maximum value of zone of coloration was obtained in TS-2 (27 mm) and minimum was detected in TS-1 (11 mm) as represented in Figure 3. Whereas, the maximum and minimum zone of coloration was found in TS-19 and TS-7 morphotypes, respectively, isolated at 50ºC. As compared to the control (no inoculation, no zone of coloration), the above results suggest that the zone of coloration were more in case of the isolates associated with the rhizosphere of Sewan grass.

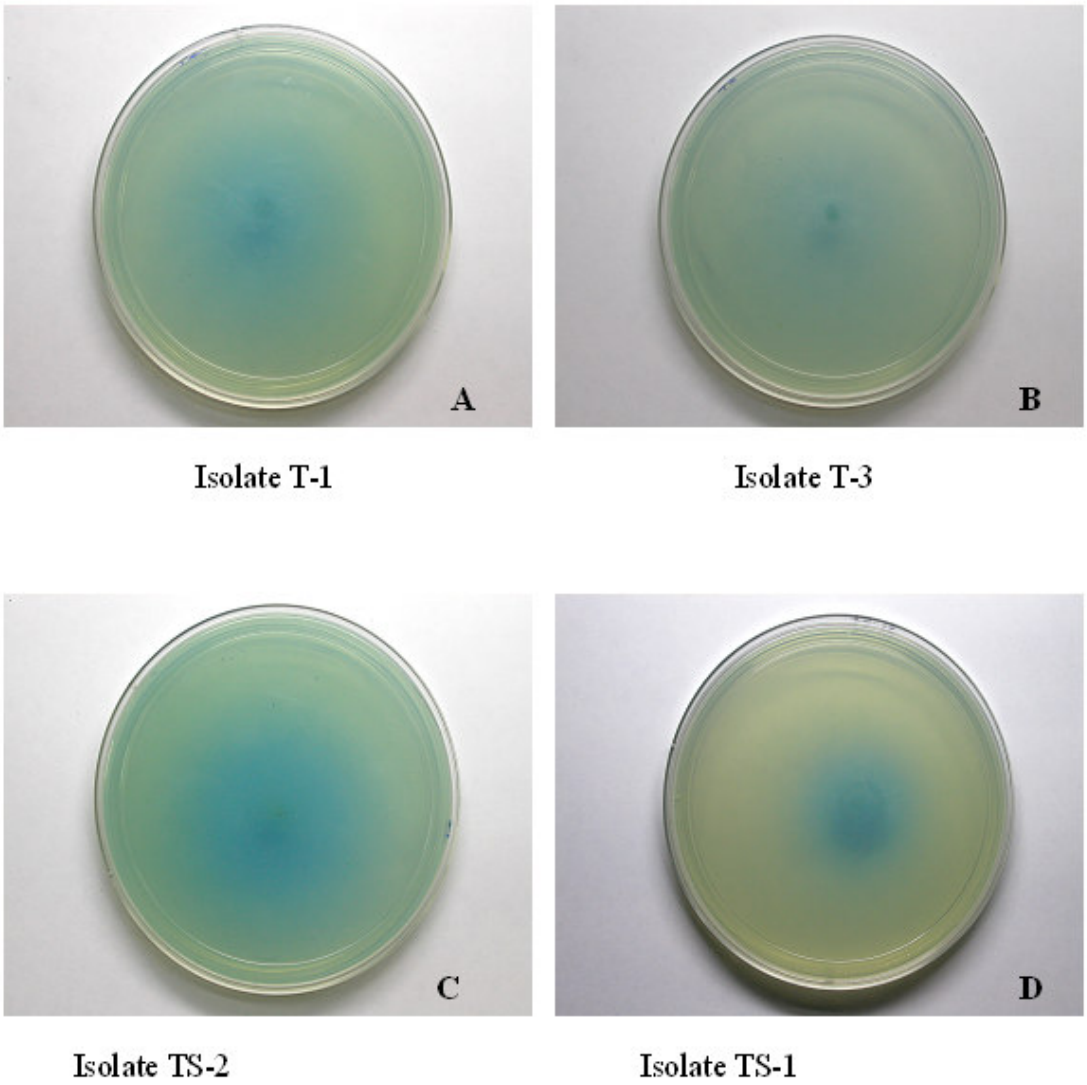


***Figure 1.*** *Maximum and minimum zone of coloration shown by the rhizosphere associated bacterial isolates of C. polygonoides (A and B, respectively) and L. sindicus (C and D, respectively) when grown at 37ºC on BTB containing N free malate agar media.*


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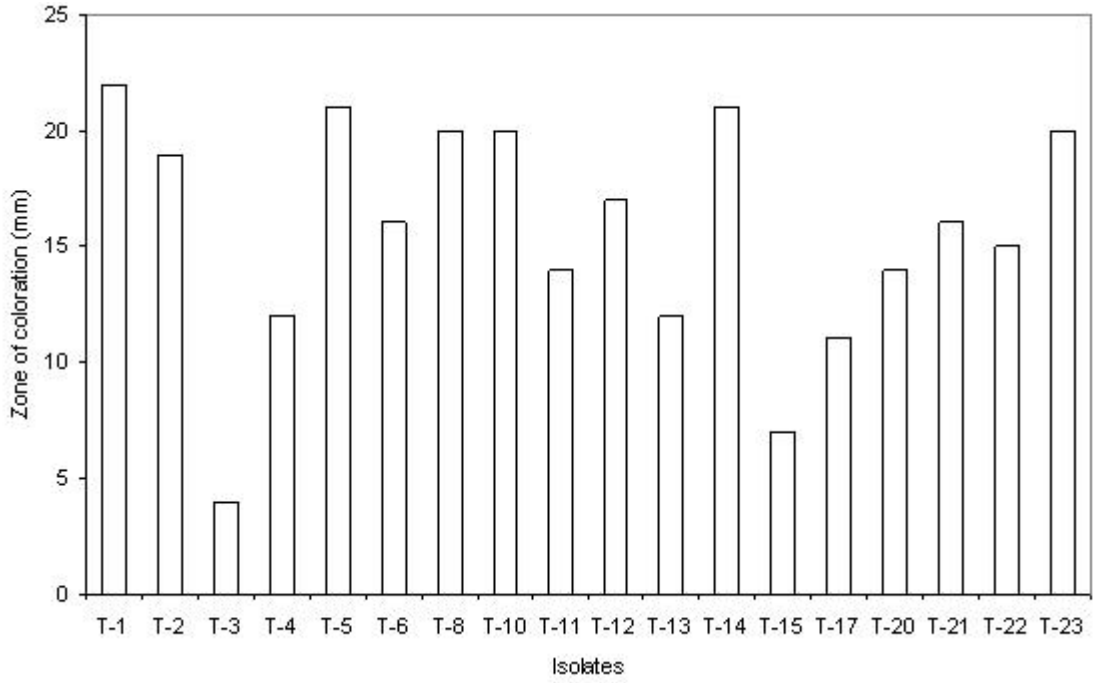


***Figure 2.*** *Zone of coloration of rhizospheric isolates associated with C. polygonoides grown at two temperatures*

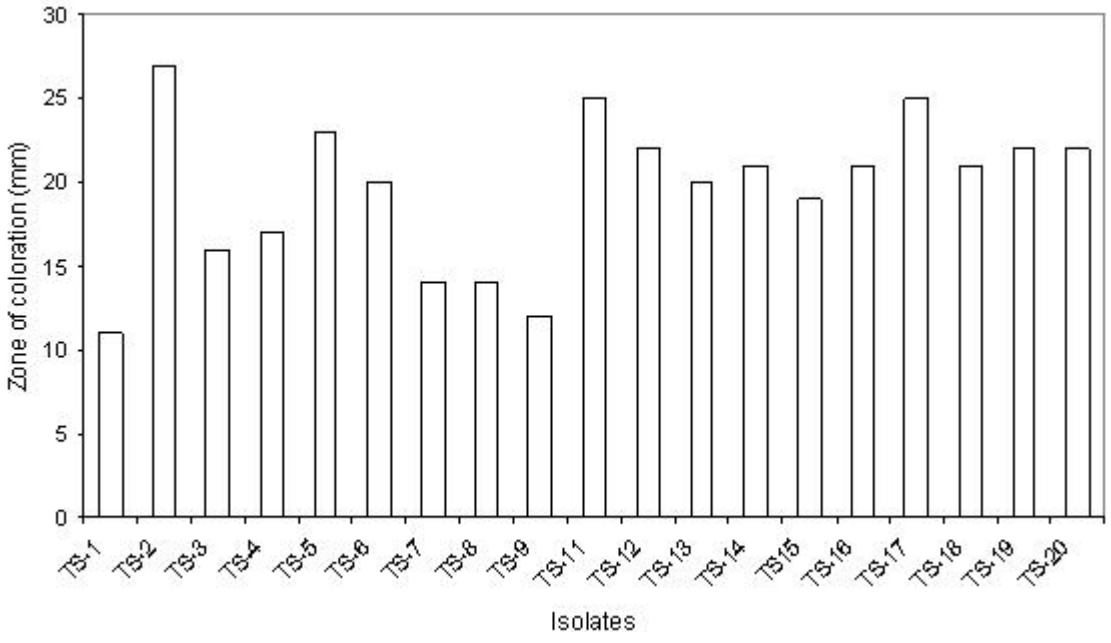


***Figure 3.*** *Zone of coloration of rhizospheric isolates associated with L.sindicus grown at two temperatures*


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### *Quantification of the nitrogen fixation*

Nitrogen fixation using all the isolates was investigated by measuring the reduction of acetylene (ARA) as described in materials and methods. It was found that some isolates which showed positive response in plate experiments, failed to show any acetylene reduction activity (ARA) in the liquid growth conditions, when analyzed by gas chromatography (T-6, 8 and all isolates of 50ºC; TS-3 and all isolates of 50ºC except TS-13). The results of the acetylene reduction data for the Phog isolates are shown in Table 1. The maximum value of the ethylene production was obtained in isolate T-5 and lowest in T-10. Similarly, the isolates TS-13 and TS-11 were identified as highest and lowest acetylene reducing isolates in case of the Sewan grass (Table 2). When the results of ARA were compared for both the plants it was found that the strains isolated from Sewan grass, were showing higher nitrogenase activity and also the number of nitrogen fixers are more in this case.

***Table 1.*** *Acetylene reduction assay of the isolates associated with the rhizosphere of C. polygonoides*

| S. No. | Isolate No. | Ethylene produced (n moles / 24 h ) |
|---|---|---|
| 1. | T-1 | 4110.5 |
| 2. | T-2 | 2265.6 |
| 3. | T-3 | 2617.5 |
| 4. | T-4 | 1763.3 |
| 5. | T-5 | 7594.8 |
| 6. | T-10 | 1657.7 |
| 7. | T-22 | 2116.5 |

***Table 2.*** *Acetylene reduction assay of the isolates associated with the rhizosphere of L. sindicus*

| S. No. | Isolate No. | Ethylene produced (n moles / 24 h ) |
|---|---|---|
| 1. | TS-1 | 5206.8 |
| 2. | TS-2 | 4080.9 |
| 3. | TS-4 | 3658.8 |
| 4. | TS-5 | 3940.3 |
| 5. | TS-11 | 1688.7 |
| 6. | TS-12 | 3236.6 |
| 7. | TS-13 | 8302.7 |
| 8. | TS-16 | 6191.8 |
| 9. | TS-17 | 3377.4 |
| 10 | TS-18 | 4362.4 |
| 11. | TS-20 | 3059.9 |


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### *Biochemical characterization of the isolates*

Various different biochemical properties of the isolates, screened from rhizosphere of both the important desert plants are given in Table 3. The strains were found both Gram-positive and Gram-negative, as depicted in the table. The bacterial colonies included in the table 3 were isolated at 37ºC except TS-13 (50ºC). This thermophilic isolate was also found to grow at 37ºC temperature (mesophilic in nature). The catalase activity was present in all isolates whereas, no isolate was found to show casein hydrolyzing, sucrose degrading activity and a negative methyl red test. The positive glucose and lactose degrading capacities were detected in T-1, T-3, TS-1, TS-18 and T-3, TS-1 isolates, respectively. Isolates T-1, TS-11 and TS-18 expressed the positive nitrate reduction and gelatin hydrolyzing activity. The maximum starch hydrolyzing activity was detected in TS-11 followed by other positive isolates. Some of the isolates (T-1, T-3, TS-1, TS-11) were also found to have the positive urease activity.

***Table 3.*** *Biochemical characteristics of nitrogen fixing bacterial isolates from the rhizosphere of two hot arid region plants*

| Plant rhizosphere | Isolate No. | Gram's reaction | Degradation of charbohydrate | | | Nitrate reduction | Gelatin hydrolysis | Catalase (3% $H_2O_2$) | Casein hydrolysis | Starch hydrolysis | Methyl red | Urease activity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Glucose | Sucrose | Lactose | | | | | | | |
| *C. polygonoides* | T-1 | + | + | - | - | + | + | + | - | - | - | + |
| | T-2 | - | - | - | - | - | - | + | - | + | - | - |
| | T-3 | - | + | - | + | - | - | + | - | - | - | + |
| | T-4 | - | - | - | - | - | - | + | - | + | - | - |
| | T-5 | - | - | - | - | - | - | + | - | + | - | - |
| | T-10 | - | - | - | - | - | - | + | - | - | - | - |
| | T-22 | - | - | - | - | - | - | + | - | - | - | - |
| *L. sindicus* | TS-1 | - | + | - | + | - | + | + | - | + | - | + |
| | TS-2 | - | - | - | - | - | + | + | - | + | - | - |
| | TS-4 | - | - | - | - | - | - | + | - | - | - | - |
| | TS-5 | - | - | - | - | - | - | + | - | + | - | - |
| | TS-11 | - | + | - | - | + | + | + | - | + | - | + |
| | TS-12 | - | - | - | - | - | - | + | - | + | - | - |
| | TS-13 | - | - | - | - | - | - | + | - | + | - | - |
| | TS-16 | - | - | - | - | - | - | + | - | + | - | - |
| | TS-17 | - | - | - | - | - | + | + | - | + | - | - |
| | TS-18 | + | + | - | - | + | + | + | - | - | - | - |
| | TS-20 | - | - | - | - | - | + | + | - | + | - | - |

### *Comparative analysis of the isolates of C. polygonoides and L. sindicus*

When all the rhizobacterial isolates from one plant were compared with another plant in terms of acetylene reduction activity, colonies isolated from *L. sindicus* were found to have a higher ARA. In case of *C. polygonoides* the isolates T-5 and T-1, showed higher ARA value (7594.8 and 4110.5 n moles / 24 h, respectively), while other isolates have ARA values in the range of 1600-2700 n moles / 24 h. In case of the morphotypes isolated from *L. sindicus*, the lowest and highest values of ARA were detected in TS-11 and TS-13 (1688.7 and 8302.7 n moles / 24 h, respectively). The other isolates have ARA values more than 3000 n moles / 24 h.

It has been reported that the concentration of root exudates in the rhizotic zones is higher in case of plants belonging to gramineae family. These exudates generally consist of carbohydrates, organic acids, amino acids and amides, vitamins and other


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compounds [10, 11]. The concentration of amino acids released by the plants are considered to be insufficient as a source of nitrogen to explain the increased microbial population in rhizosphere [18] indicating that nitrogen may be limiting for microbial growth in the rhizosphere, giving nitrogen fixers a potential advantage [2].

## Conclusions

Various plant developmental processes are controlled by internal signals that depend on the adequate supply of mineral nutrients by soil to roots. Therefore, the availability of nutrient elements can be a major constraint to plant growth in many environments of the world, particularly in the hot arid areas where the soils are extremely low in nutrients. The potential of non-symbiotic N fixation can provide an improved solution in these areas to promote plant development. A better awareness and understanding of the role of non-symbionts in these areas would then be helpful in the development of bio-inoculum. The approach of developing the bio-inoculum for the studied areas looks appealing as the fertility of the soil may be increased by inoculating the best suited bacterial strain which intern will give rise to a better plant population in the natural conditions without applying fertilizer input from out side.

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# ECOSYSTEMS AS CLIMATE CONTROLLERS – BIOTIC FEEDBACKS

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**Abstract**. There is good evidence that higher global temperature will promote a rise of green house gas levels, implying a positive feedback which will increase the effect of the anthropogenic emissions on global temperatures. Here we present a review about the results which deal with the possible feedbacks between ecosystems and the climate system. There are a lot of types of feedback which are classified. Some circulation models are compared to each other regarding their role in interactive carbon cycle.
**Keywords:** *climate control, climate change, biotic feedbacks, ecosystem, community*

## Introduction and aims

It is obvious that human activities could affect the weather and the climate of the Earth, and could change the chemical content of the atmosphere. There is a continuous cycle and flow of inorganic compounds between the atmosphere and the ecosystems, therefore the anthropogenic affects (such as $CO_2$ emission) strongly modify the activities of ecosystems. The modified activities are as follows: fluxes of the photosynthesis, $CO_2$ emission of the soil or the quantity of dissolved organic compounds in the ocean. These activities could have a feedback on the climate controlling the compounds of the atmosphere and therefore on the temperature of the Earth.

The latest IPCC report (*Fischlin et al., 2007*) points out that a rise of 1,5-2,5 $^oC$ in global average temperature makes important changes in the structure and in the working of ecosystems, primarily with negative consequences towards the biodiversity and goods and services of the ecological systems.

The ecosystems could control the climate (precipitation, temperature) in a way that an increase in the atmosphere component (e.g. $CO_2$ concentration) induces the processes in biosphere decrease the amount of that component through biogeochemical cycles. Paleoclimatic researches have proved this control-mechanism for more than 100,000 years. The surplus $CO_2$ content has most likely been absorbed by the ocean,


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thus controlling the temperature of the Earth through the green house effect. This feedback is negative therefore the equilibrium is stable.

During the climate control there may be not only negative but positive feedbacks. One of the most important factors affected the temperature of the Earth is the albedo of the poles. While the average temperature on the Earth is increasing the amount of the arctic ice is decreasing. Therefore the amount of the sunlight reflected back decreases, which warms the surface of the Earth with increasing intensity. This is not the only positive feedback during the control, another good example is the melting of frozen methane hydrate in the tundra.

The most important circle in the biosphere is the global carbon cycle; the changing of the goods, services of ecosystems affected by human interference are examined. This article deals with the ecosystem-climate control and feedbacks.

There are some models in the great climate centres where the composition of the atmosphere is calculated considering the anthropogenic effects with respect to the future. During modelling the control is partly examined, but the climate models are different. In this article the climate-biosphere interaction models are compared regarding to the feedbacks.

## Working and modelling of the ecosystems

### *Organization of ecological systems*

An ecosystem is a natural unit that includes all plants, the non-living physical factors and their relations to each other (*Pásztor et al., 2007*). Functions of ecological systems are affected by abiotic environment and biotic factors. The abiotic environment consists of non-physical ecological factors which are the physical and chemical properties of the soil, topographic (e.g. elevation) and climatic components (*Moser et al., 1992*). Biotic factors are the interactions of organizations (producer, consumer and demolisher organizations), the anthropogenic factors could occur directly and indirectly. Indirectly means a way where the physical, chemical, biological conditions are changed, on the other hand, directly means the effect toward the living creature (e.g. deforestation).

The dynamic unity of habitat and biome is called ecosystem which has a well-defined energy circulation. The ecosystem's components could be exchanged therefore it is called an opened system. The highest ecosystem is the biosphere where creatures live.

Every ecosystem is characterized by defined assortment and number of species. The ecosystem has not only spatial but temporal expansion, it is in equilibrium, the work of nutrition and energy chain is continuous.

#### *Ecosystem services*

Ecosystem services are functions that are enjoyed by mankind. These services can be collected into a few groups (*MEA, 2005*):

- Provisioning services: the ecosystems take part in producing various products (e.g. food, water, wood)
- Regulating services: climate control, carbon stocks, reconstruction after disaster
- Cultural services: these give aesthetic, spiritual experiences
- Supporting services: primary and secondary production, maintenance of biodiversity, biogeochemical circles


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The connection between ecophysiological properties of the plants and the ecosystem's role are in *Table 1*.

***Table 1.*** *The connection between ecophysiological properties of the plants and the ecosystem's role (Buchmann, 2002)*

| Ecophysiological properties of the plants | Ecosystem's role |
|---|---|
| Photosynthesis, respiration and evaporation | Net Ecosystem Exchange of $CO_2$ and $H_2O$ |
| Dispersion of carbon (above and under surface of the land), competition between species, symbiotic connections | Dispersion of fluxes of ecosystems |
| Growth, aging, falling of leaves | Carbon source and sink |
| Mineral resources, climate, life form, plant functional types, phenology, structure of foliage, succession | Spatial and temporal fluctuation between and in the ecosystems |

*Energy flux*

The basis of the human existence is the photosynthesis and the only energy source is the Sun. Half of the incoming solar radiation ( 342 W/m$^2$) reaches the surface of the Earth (168 W/m$^2$). A little part of this radiation is utilized in photosynthesis (the photosynthetically active radiation, $\lambda$=400-770 nm) and the solar energy changes to chemical energy during the photosynthesis:

$$6\,CO_2 + 6\,H_2O \xrightarrow{Light,\,plant} C_6H_{12}O_6 + 6\,O_2 \qquad \Delta G' = +2850\ kJ \qquad \text{(Eq.1)}$$

$\Delta G'$ is the change of the standard enthalpy which can be used for profitable work in the plant. The plants convert 1 % of the incoming solar radiation into chemical energy; this amount maintains the work of the biosphere.

There are three types of photosynthesis according to the bond of the $CO_2$:

- $C_3$ photosynthesis: the $CO_2$ is first incorporated into a 3-carbon compound. Photosynthesis takes place throughout the leaf. Most plants are $C_3$.
- $C_4$ photosynthesis: the $CO_2$ is first incorporated into a 4-carbon compound. Photosynthesis takes place in inner cells. $C_4$ plants include several thousand species in at least 19 plant families. Example: forewing saltbush, corn, and many of our summer annual plants.
- CAM photosynthesis: the $CO_2$ is stored in the form of an acid before it is used in photosynthesis. CAM plants include many succulents such as cactuses and agaves and also some orchids and bromeliads.

Some properties of the various photosynthesises are compared in *Table 2*.

***Table 2.*** *Comparison of $C_3$, $C_4$ and CAM photosynthesises (Hopkins, 1999)*

| Property | $C_3$ | $C_4$ | CAM |
|---|---|---|---|
| Theoretical energy demand ($CO_2$:ATP:NADPH) | 1:3:2 | 1:5:2 | 1:6,5:2 |
| Transpiration ratio (g $H_2O$/g dry mass) | 450-950 | 250-350 | 18-125 |
| Speed of photosynthesis (mg bonded $CO_2$/(dm$^2$h)) | 15-30 | 40-80 | (low) |
| Optimum of the temperature ($^0$C) | 15-25 | 30-47 | 35 |


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*Biogeochemical cycles*

20 chemical elements take part in the structure of the living creature. Some of them appear in large amount (C, N, P, H, O), others in small quatities (S, K, Na, Ca, Mg, Fe, etc.).

The various biogeochemical cycles exist for every element but they are not separated from each other. The material makes continuous cycling (*Szabó I.M, 1989*):

- Carbon cycle
  In food chain carbon almost always exist as $CO_2$. During the carbon cycle the carbon goes on through the food chain in organic bond and leaves for the atmosphere as $CO_2$. The methane oxidizes fast into $CO_2$, the carbon of the geological sediment are solved by erosion.
- Water cycle
  The hydrological cycle are maintained by the solar energy and the gravitation. More than 80% of the total insolation evaporate water. The atmospheric water vapour condensates and because of the gravitation it falls down like moisture. The energy used during the evaporation process disperses in the atmosphere like heat.
  Almost 95% of the water in the Earth is in rocks bonded chemically and physically, does not circulate. 97,3% of the rest is in the oceans, 2,1% is in polar ice and glaciers and the rest is in fresh water (atmospheric water vapour, inland waters, groundwater). The speed of the water cycle between the surface and the atmosphere is very fast, the total cycle is repeated 32 times a year.
- Nitrogen cycle
  The largest amount of the nitrogen is in the air but most of the plants are not able to use it directly, it is done by nitrogen-bonded bacteria. The ammonia from the bonded nitrogen becomes nitrates by some other bacteria. These are water soluble feeds for any plant. The nitrates that are not used are converted into nitrites and nitrogen.
- Phosphorus cycle
  During the phosphorus cycle the water solves the phosphates from the rocks. The phosphorus is taken up by the plants from aqueous solution of inorganic compounds, the animals take up by eating plants. The bacteria deliver inorganic compounds that contain phosphorus from dead beings. The solved phosphorus from the soil goes into the ocean through fresh water, it gets into fishes, and then through birds of prey get into the mainland as guano.
- Cycle of other biogen elements
  There are important structural and regulating role of the Ca cycling, the sulphur is an important component of some amino acid.

The natural material fluxes of the ecosystems are disturbed by human activities. There are some compounds and radioactive substance that have damaging effect (Sr-90, mercury, DDT, etc.).

***Earth-climate interactions***

Determining feature of the behaviour of the climatic status is the free variability done by nonlinear inner feedbacks. If the concentration of the carbon dioxide redoubles in a hypothetic atmosphere devoid of feedbacks then $+4 Wm^{-2}$ radiative forcing will be put out, which means an 1,2 °C increase in temperature (*Götz, 2004*).


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The equilibrium climate sensitivity is the change of global surface average temperature in a year in case of redoubling the atmospheric concentration of carbon dioxide (*Hegerl et al., 2007*). If the equilibrium conditions are not realized then it is called effective climate sensitivity. The climate sensitivity parameter shows how much change in the global surface average temperature in a year is caused by one unit change in radiative forcing (measure unit: $^{\circ}C(Wm^{-2})^{-1}$ ).

According to the IPCC AR4 report the equilibrium climate sensitivity will likely (more than 66% probability) be between 2 $^{o}$C and 4.5 $^{o}$C, the best estimation is at about 3 $^{o}$C. It is unlikely to be under 1.5 $^{o}$C.

Paleoclimatic research shows that the change in temperature is in proportion with the concentration of the $CO_2$ in the atmosphere during the last 220,000 years (*Barnola et al., 1987*). The amount of the methane and the temperature are in close relationship (*Raynauld et al., 1993*). The ratio of the temperature and $CO_2$ concentration is different in various periods. Considering the last 220,000-year period the amount of $CO_2$ increase was 100 ppm, the increase in temperature was 8 $^{o}$C, which equals to 25 PgC/$^{o}$C rate. This sensitivity of the temperature was 40 PgC/$^{o}$C during the little ice-age (1660-1900), this value is 6 PgC/$^{o}$C nowadays (1958-1992) (*Woodwell et al., 1998*).

The main element of the controlling system is the feedback loop. If there is no feedback loop, then the whole effect related to the climate can be calculated from the outer effects:

$$Y=X, \qquad \text{(Eq.2)}$$

where $Y$ is the output parameter, $X$ is the input parameter. If there is one feedback loop, the equation is as follows:

$$Y = X + g \cdot X = (1+g)X \qquad \text{(Eq.3)}$$

where $g$ is the gain parameter. If there is an infinitive number of repeats, then:

$$Y = X\left(1+g+g^2+g^3+...\right)=\frac{X}{1-g}, \text{ if } g<1. \qquad \text{(Eq.4)}$$

If $1>g>0$, then it is called positive feedback, if $g<0$, then it is negative feedback.

If $g>1$, then the process is instable, $Y=\infty$.

During the modelling process the general circulation models that are used contain some important feedback mechanisms (*Lashof et al., 1997*). The most important is the positive feedback of the ice albedo; the second positive feedback is related to the amount of the water vapour. The third feedback is uncertain in its size and direction; this is the effect of the clouds. The gain of these three feedback loops ($g$) is between 0,4-0,78 values.

*Classification of the feedbacks*

The feedbacks can be classified in accordance with their effects (*Hartman et al., 2003*):

- Effect to the amount of the climate change
  - Cloud, water vapour feedbacks
    The increased $CO_2$ concentration heats the air directly due to the green house effect which takes up more water vapour with higher temperature. Therefore the heat absorbing ability increases and more uptake of water vapour is caused. The increased amount of the water content of the air-strata could also cause negative feedbacks. The infrared radiation is absorbed by the clouds and heating effect is caused in the rate of the absorbed dose. At the same time the sunshine is reflected back so the


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warming is blocked. (*Hegerl et al., 2007; Ammann et al., 2007*). The degree of the feedback caused by water vapour is difficult to calculate, because the water vapour -in spite of $CO_2$- does not dissipate evenly in the air.

- Ice-albedo feedback
  The global warming heats the surface of the Earth, melts the ice barriers. The white surface of the ice reflects the solar radiation and during its melting the heat is absorbed by the oceans. For this reason the ice-surfaces melt faster and a self-exciter process evolves. (*Soden et al., 2006*)
- Biogeochemical feedbacks and the carbon cycle
  The global carbon cycle and the sulphur cycle contain important feedbacks as well. For example, increase in the concentration of $CO_2$ affects the heat-absorbing ability of the soil. The carbon content of the soil is stored in a sensitive equilibrium, so little change in the temperature is enough for the soil to emit the absorbed $CO_2$.
  The transpiration of the plants in rainforests is heightened by the increase of the carbon dioxide concentration. When the stomas are open during the transpiration process a part of their water content is evaporated.
  Another positive feedback process is the methane emission from the methane hydrate related to the global warming. Methane hydrate is solid but instable which is originated in the deep sea on low temperature, under high pressure. The basic condition of the formation of methane hydrate is thick sediment in which the methane is formed. If this material comes up to the surface of the oceans then it directly sublimates and accelerates the process of global warming through green house effect.
- Atmospheric chemical feedbacks
  The presence of the aerosols decrease the surface temperature of the Earth by 2-3 $^{o}$C, larger cooling could be felt above the industrialized area, because half of the aerosols in the atmosphere has anthropogenic origin.
  The chemical processes of the stratosphere and the troposphere are in contact with temperature, precipitation, circulation and the content of the atmosphere, so the radiation equilibrium of the Earth is affected by them.

- Effect to the transient reaction of the climate
  - Heat uptake of the oceans and the circulation feedbacks
    Warming of the sea and the above air-strata could heighten the evaporation; i. e. it could increase the water content of the air. Water vapour is the most effective green house gas. If the concentration of the green house gases increases, then warming occurs, the humidity of the atmosphere increases and strengthens the green house effect.
    The relation between the atmospheric $CO_2$ and the North Atlantic Deep Water has positive feedback as well.
- Effect to the pattern of the climate change
  - Hydrological and vegetation feedbacks
    These are soil-water feedback, snow-albedo feedback, density of stomas, leaf area feedback, bio-geographic feedbacks.
  - Natural variations in the climate system


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The El Nino anomaly is caused by the interaction between the atmospheric $CO_2$ and the ocean around the Equator. The feedback between the air, ocean and the atmospheric $CO_2$ concentration is positive. (*Geresdi et al., 2004*)

### *Global carbon cycle*

*Carbon cycle with natural and anthropogenic effects*

The only carbon source of the organic substance of living beings is the atmospheric $CO_2$ gas or the solved $CO_2$ in oceans and waters. This kind of $CO_2$ converts from inorganic substance to organic substance during the photosynthesis.

The atmospheric $CO_2$ concentration has fluctuated between 180 ppm and 300 ppm for 650,000 years according to glacial and interglacial terms. It is generally accepted that the $CO_2$ left for the atmosphere was absorbed by the ocean during the glacial maximum time. Until 1750, the $CO_2$ amount was between 260 and 280 ppm for 10,000 years and the anthropogenic fluctuation of the carbon cycle was negligible compared to the natural effects. The concentration has been continuously increasing since 1750; the last measured value was 380 ppm in 2005. The human activities, the increase of $CO_2$ concentration caused by, consist of:

- Burning of fossil fuels
  The world's whole oil use was $169{,}362 \cdot 10^{15}$ Btu in 2005, gas use was $107{,}613 \cdot 10^{15}$ Btu and coal use was $122{,}562 \cdot 10^{15}$ Btu (1Btu (*British Thermal Unit*)=1054-1060 J), These values are the equivalent to 5,000 Tg C/year amount. (*EIA, 2006*)
- Deforestation
  Forests are situated on the 30.3 % of the Earth's area (39,520,630 $km^2$). The change was
  -73,170$km^2$/year between 2000 and 2005 (*FAO, 2007*). An amount of 1000 Tg C/year goes up to the atmosphere.
- Cement production (*Worrell et al., 2001*)
  In 2005 2284 Tg cement was made while 307 Tg C was emitted. From this 160 Tg C evolved during the producing process and 147 Tg C originated from the energy use.
- Land use change (biomass burning (*Ito et al., 2004*), industrial growing, converting grass lands into agricultural area)
  The biomass burning was 5613 Tg dry substance in 2000: 2814 Tg dry substance is assigned to the opened flames, the rest of these is to the burning of bio-fuels. Altogether, 2290 Tg C evolved as $CO_2$ and 32.2 Tg C evolved as methane. *Houghton (2006)* has examined the size of the land use changing and has found that the emission resulted from the annually change was 2.18±0.8 Pg C/year between 1990 and 1999.

The concentration of the atmospheric methane has an analogous increase. The $CH_4$ concentration was 700 ppb in 1750, while it became as much as 1775 ppb in 2005. The primary sources of this increase are the following ones: (the values related to 1990s, *Stern et al., 1998*)

- Fossil fuels: 15.2 Tg $CH_4$ (gas burning), 18.0 Tg $CH_4$ (gas supply), 46.3 Tg $CH_4$ (coal mining)
- Moulding the ground: 40.3 Tg $CH_4$


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- Peat land, wetland: 200 Tg $CH_4$ (*Wang et al., 2004*)
- Ruminant animals: 113.1 Tg $CH_4$
- Rice cultivation: 100.8 Tg $CH_4$

Both $CO_2$ and methane play an important role during the global carbon cycle, their continuous fluxes are present among the ocean, the terrestrial biosphere and the atmosphere.

According to the IPCC AR4 report (*Fischlin et al., 2007*) the carbon flux between the terrestrial biosphere and the atmosphere is 120 Gt C/year (*1 Gt = 1Pg = 1000 Tg*); it is 70 Gt C/year between the ocean and the atmosphere , considering the natural effects. Regarding to the anthropogenic effects, the flux between the ocean and the atmosphere increases to 90 Gt C/year.

The increase of the $CO_2$ concentration is 3.2±0.1 Gt C/year in the 1990s. (The rate of increase has become 4.1±0.1 Gt C/year between 2000 and 2005.) The emission is 6.4 Gt C annually due to fossil fuels and cement production, 1.6 Gt C/year due to the land-use change. The ocean takes up 2.2 Gt carbons annually and the terrestrial uptake is 2.6 Gt C/year.

*Ocean carbon cycle*

One of the absorbent of the $CO_2$ emitted to the atmosphere is the ocean. The ocean slowly reacts to the change of the $CO_2$ content of the atmosphere due to the slow mixing. The ocean $CO_2$ uptake could be decreased by the global warming because the rate of the $CO_2$ dissolution is decreased by the warming of the water-layers close to the surface. The gas dissolves easier in cold water than in warm water, but it dissolves easier in salted sea-water than in pure water because the oceans contain carbonate ions.

There are three types of carbon content in the ocean. The largest amount (98.1%) is in the form of dissolved inorganic compound (DIC), the second (1.85%) is the dissolved organic compound (DOC) and in 0.05% as organic particle (live or dead, particulate organic carbon, POC) (*Hegerl et al., 2007*). The proportions of the carbon-forms show that abiotic interactions and feedbacks decisively exist in the ocean.

There are three biological and physical pumps for $CO_2$ inside the ocean carbon cycle:

- Dissolving pump
  Hydrogen-carbonate arises during the reaction of carbon dioxide and carbonate ion. Because of this reaction the inorganic carbon exists in 0.5 % rate as $CO_2$ gas in the ocean. Since the amount of the $CO_2$ gas is low in the seas, more carbon dioxide could be taken up by the ocean. If the water stays on the surface and warms up, the carbon dioxide comes quickly back to the atmosphere. But if the water delapses in the ocean, the $CO_2$ could be stored thousand years before the circulation makes it come up to the surface. On high latitudes the water of the South Ocean, the Labrador Sea and the North Sea delapses into deepwater. These areas are the most important $CO_2$ sinks of the ocean.
- Organic carbon-pump
  The $CO_2$ is taken up by phytoplankton during the photosynthesis and the phytoplankton is eaten by bacteria. Therefore the nutritive and the carbon dioxide come back to the water. This process is the remineralisation which occurs especially in the surface waters. If the phytoplankton die and delapse to the deep water, the $CO_2$ is taken up during the remineralisation and is stored decreasing the effect of the global warming. This type of $CO_2$ delapse happens on high latitudes


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because there exist much phytoplankton there, so a great amount of $CO_2$ could sink to the deep water layers.

- $CaCO_3$ dissolving pump
  $CO_2$ is bonded by the third pump in the shells and corals. For the first sight it seems that much $CO_2$ is bonded in the form of limestone, but the limestone evolves carbon dioxide gas. The reason is the equilibrium where from two hydrogen-carbonate ions become $H_2O$, $CO_2$ and carbonate ion. The deep water layers are alkaline which is good for the dissolving of the limestone; while surface water layers are acidic which is for the evolving the $CO_2$ gas. The corals live in the warm waters so the evolving gas reaches the surface fast and goes to the atmosphere.

*Terrestrial carbon cycle*

The net carbon exchange is the difference between the $CO_2$ uptake by the photosynthesis, the respiration of the plants, soil and emissions of other processes (fire, wind, insect attack, deforestation, land use changing). In the last 30 years this net carbon flux was -1.0±0.6 GtC. The processes affected by the carbon cycle in the terrestrial ecosystems are as follows:

- Direct climate effects (precipitation, fluctuation of the temperature): for example the soil respiration increases with increasing temperature
- Change in the composition of the atmosphere ($CO_2$ fertilization, food accumulation, pollution)
  There is no agreement among the researchers if the net primer production (NPP) increases due to $CO_2$ fertilization (2/3 of the experiments has proven this). The effect of the fire is the largest to the flux between the terrestrial ecosystems and the atmosphere (biomass and soil become $CO_2$), 1.7-4.1 Gt C/year is the oxidation rate by fires, this is the 3-8% of the terrestrial NPP.
- Effects due to land-use change (deforestation, afforestation, agricultural exercises, their legacy)

*Missing sink*

The environment is polluted by burning fossil fuels or related to land-use change due to human activities which are important $CO_2$ sources. The $CO_2$ evolves to the atmosphere, dissolves to the oceans, is taken up by the boreal forests and a part of it is missing. (*Woodwell et al., 1998*; *Hegerl et al., 2007*). The equilibrium (related to 1990) is that as follows:

| Sources | | | | | Sinks | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Fossil fuels | + | Land-use change | = | Atmosphere | + | Boreal forests | + | Ocean | + | Missing sink |
| 6.4±0.4 | | 1.6±1.1 | | 3.2±0.1 | | 0.6±0.6 | | 2.2±0.4 | | 2.0±1.1 |

*$CO_2$ flux between the atmosphere and the biosphere in Hungary*

The residence time of $CO_2$ in the atmosphere is quite long to mix evenly in the whole troposphere of the Earth. Its concentration is approximately the same everywhere except above the source areas, there is little difference between the more polluted northern and southern hemisphere (*Haszpra, 2000*).


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*Haszpra and Barcza(2001)* show, that the net carbon dioxide uptake was 4.85 t in 1998 and 3.38 t in 1999. The measurement point is in 82 m above the land surface which is related to the nearest 200 $km^2$ area. The anthropogenic emission was 60 million t $CO_2$ (16.4 Mt C) in 2004 (*UNFCCC, 2006*). The Hungarian ecosystems were net emitters between 2002 and 2006 (in average annually 8 Mt $CO_2$) (*Barcza et al., 2008*).

*$CO_2$ flux data of ecosystems and carbon storing capacity*

The various ecosystems take part in the maintenance of the global carbon cycle in several ways. Summarizing the area of the different ecosystems, their carbon storing capacity and NPP values are found in Table 2.

***Table 3.*** *Size and NPP values of different ecosystems*

| Ecosystems | Area [M $km^2$] | Carbon storage | NPP (MEA, 2005) [kg C/$m^2$/year] |
|---|---|---|---|
| Deserts | 27.7 | * | 0.01 |
| Steppes and savannas | 40<br>Tropical: 28 ($C_4$)<br>Temperate: 15 ($C_3$/$C_4$) | * | <br>0.34<br>0.49 |
| Seaside terrestrial ecosystems | 6 | * | 0.52 |
| Forests | 41.6<br>Tropical: 17.1<br>Temperate: 10.4<br>Boreal: 13.7 | 1640 PgC | 0.68 |
| Tundra and arctic regions | 5.6 | 400 PgC<br>(Gruber et al., 2004) | 0.06 |
| Mountains | 35.8 | * | 0.42 |
| Freshwater, wetland | 10.3 | 450 PgC (wetland) | 0.36 |
| Oceans, seas | 349.3<br>(14 billiard $km^3$) | 38100 PgC<br>(of it 698-708 PgC organic and 13-23 PgC biomass) | 0.15 |

*: There is no data.

## *Modelling*

The expected changes in climate system could be predicted with segmentalized units of the Earth during simulations. In this respect the parts of the biosphere consist of atmosphere, ocean, behaviour of plants and anthropogenic effects. The atmospheric and the oceanic conditions could be described by the general circulation models (GCM), the vegetation models describe the behaviour of the plants and the forecastcontaining


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anthropogenic effects is called emission scenarios. With the collective using of these models it is predicted to long time range what changes are expected in our planet life. The new generation models are the Earth System Models of Intermediate Complexity (EMIC) which simulate the operation of the Earth.

*Emission scenarios*

The Intergovernmental Panel of Climate Change published the SRES report (Special Report on Emission Scenarios) related to the anthropogenic emission states (*IPCC, 2000*). Altogether 40 different SRES scenarios were made in all which were classified into 6 groups according to the various social and economic effects. These categories are the illustrative SRES scenarios which include the following conditions:

- *A1* describes a future world with very rapid economic growth, global population that peaks in the mid-century and declines thereafter, and the rapid introduction of new technologies.
  - *A1FI*: intensive fossil fuels
  - *A1T*: non fossil energy sources
  - *A1B*: balance across all sources
- *A2* describes a very heterogeneous world in which the main purpose is the self-reliance and the preservation of the local identities. The fertility patterns slowly converged. The economic development is regionally oriented.
- *B1*describes a convergent world with *A1* population, but the economical structure is rapidly changing.
- *B2* emphasizes the local solutions to economic, social and environmental sustainability. The growth of the population is increasing like in *A2* case, but slower.

The SRES scenarios do not contain more emission-decreasing initiatives but the double aerosol effect is considered; it is calculated with human (e.g. industry, heating, traffic) and natural (e.g. sea salt) effects.

*General Circulation Models (GCM)*

The general circulation models describe the movements in three dimensional space. Two types of models exist: the atmospheric (AGCM) and the oceanic general circulation model (OGCM). The AGCM is similar to the weather forecast but the predictions are not in days but in decades or even centuries. Connection between the surface of the Earth and the cryosphera is examined by atmospheric circulation models in three dimensional space. The oceanic circulation model consists of the description the ocean and sea-ice where ocean turbulence, temperature and concentration circumstances are considered.

At the end of the 1960s the atmospheric and the oceanic models are coupled into Atmospheric-Oceanic General Circulation Models (AOGCM) (*Bryan, 1969; Manabe, 1969*). More well-known AOGCMs (*Randall et al., 2007*) can be found in *Table 4.*


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***Table 4.*** *Coupled atmospheric- oceanic general circulation models and their place related to IPCC AR4 (the numbers are related to web references)*

| Model (AOGCM) | Country | Model (AOGCM) | Country |
|---|---|---|---|
| BCC-CM1 | China [1] | FGOALS-g1.0 | USA [9] |
| BCCR-BCM2.0 | Norway [2] | GFDL-CM2.1 | USA [10] |
| CCSM3 | USA [3] | GISS | USA [11] |
| CGCM3 | Canada [4] | INM-CM3.0 | Russia [12] |
| CNRM-CM3 | France [5] | IPSL-CM4 | France[13] |
| CSIRO-MK3.0 | Australia[6] | MIROC3 | Japan [14] |
| ECHAM5/MPI-OM | Germany [7] | MRI-CGCM2.3.2 | Japan [15] |
| ECHO-G | Korea, Germany [8] | UKMO-HadCM3 | Great Britain [16] |

*Vegetation modelling*

The dynamic of the biosphere is described by different vegetation models. The vegetation of the ocean is more simple, therefore the biogeochemical model of the ocean is built in the OGCMs, these are for example OPA, MIT (*Peylin et al., 2005*). The biogeochemical part consists of the dynamic of plankton and the flux between the ocean and the atmosphere.The dynamic of terrestrial ecosystems are more complex. The processes can be classified into three groups related to their speed; the fastest is the photosynthesis and the respiration, the medium rate changes are processing during the life cycle or season by season and the slowest are the evolutional changes in the genetic structure of organizations. Global vegetation models are developed to connect the vegetation and the climatic conditions interactively (*Foley et al., 1996*). This connection was based on an asynchronous equilibrium; the models (climate and vegetation) could approach each other with iteration resulting long calculations. The other types of the vegetation models are the Dynamic Global Vegetation Models (DGVM) which simulate transient vegetation dynamic. The changes in the function of the ecosystem (water-, energy- and carbon-balance) and the structure of the vegetation (distribution, physiognomy) are calculated by every model to the effect of the results of the various circulation models. There exist some DGVM and their place of development is shown in *Table 5*.

***Table 5.*** *Some Dynamic Global Vegetation Model*

| Model (DGVM) | Place of development |
|---|---|
| TRIFFID (Top-down Representation of Interactive Foliage and Flora Including Dynamics) | Hadley Centre, Great Britain |
| LPJ (Lund Potsdam Jena Dynamic Global Vegetation Model) | Potsdam Institute, Germany |
| HYBRID | LSCE, France |
| IBIS (The Integrated Biosphere Simulator) | SAGE, USA |
| SDGVM (Sheffield Dynamic Global Vegetation Model) | CTCD, Great Britain |
| VECODE (Vegetation Continuous Description) | Potsdam Institute, Germany |
| MC1 | VEMAP, USA |
| CLM (Community Land Model) | NCAR, USA |


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In vegetation models there are the following key processes:

- Physiological features : photosynthesis, respiration, stoma conductivity, nutrient uptake
- Structure of the ecosystem: partitioning and growth, phenology, reproduction
- Dynamic feature of vegetation: competition, herbivore, fire and illness, mortality

For modelling the terrestrial carbon cycle a TRIFFID vegetation model was developed by *Cox et al., 2001*. The net carbon flux of the biosphere is defined by the difference between the $CO_2$ emission related to respiration and uptake related to growth. In the long run the biosphere is in equilibrium, the amount of the stored carbon is stable. For a short period there is obviously no equilibrium because of daily, seasonally and annual changes. This equilibrium is influenced by the climate change and anthropogenic emissions. The main elements of the TRIFFID model are:

- Five plant functional types: broadleaves, needle leaves, shrubs, $C_3$ and $C_4$ grass
- Newly originated organic substance (BPP, GPP) and the respiration are defined for every PFT. These parameters depend on climatic conditions (temperature, soil moisture). The difference between them is the net primer production (NPP). This C-content gets to soil through roots, fallen leaves where it is demolished by microbes and gets back to the atmosphere by soil respiration. The difference between NPP and soil respiration is the net ecosystem production (NEP); this amount is stored (positive balance) or released (negative balance) by the biosphere.

*Earth System Model of Intermediate Complexity (EMIC)*

Development of EMIC models was made in the frame of International Geosphere-Biosphere Program (*Claussen et al., 2002*). The summarized models can be seen in *Table 6-8 (Claussen, 2005).* Among the listed EMIC models the Climber, GENIE, ISAM-2, LOVECLIM and UVic models are suitable for the biotic interactions.

***Table 6.*** *Description of EMIC models*

| EMIC models | Main purpose |
|---|---|
| Bern 2.5D [17] | Examination of ocean thermohaline circulation |
| Climber-2 [18] | Long term simulations over several millennia |
| Climber 3α[18] | Investigation the role of the ocean |
| GENIE [19] | Computationally efficient models both the paleo, future, Earth system models |
| IAP RAS [20] | Simulation of large scale processes |
| ISAM-2 [21] | Detailed carbon and methane cycle, simplified ocean dynamics |
| LOVECLIM [22] | The coupling of global carbon cycle and the climate for decades to millennia |
| McGill Paleoclimate model-2 [23] | Long term simulations for Paleoclimate investigations |
| MIT IGSM [24] | Examination of anthropogenic effects with the vegetation feedbacks for short term |
| MoBidiC [25] | Milankovitch's astronomical theory and climate feedbacks on time scale of several million years |
| Planet Simulator [26] | For Paleoclimatic investigations |
| PUMA [27] | Simulation on long time scales and inexpensive hardware |
| UVic [28] | With feedbacks of the global carbon cycle on time scales 10-1000 years |


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***Table 7.*** *Details of EMIC models*

| EMIC Model | Atmosphere | Ocean | Sea ice | Atmosphere-land contact | Ocean biosphere |
|---|---|---|---|---|---|
| Bern 2.5D | + | + | + | - | + |
| Climber-2 | Potsdam-2 | MUZON | + | ASI | + |
| Climber 3α | Potsdam-3 | GFLD | + | ASI | + |
| GENIE | IGCM+EMBM | GOLDSTEIN | + | - | BIOGEM |
| IAP RAS | + | + | + | + | - |
| ISAM-2 | + | + | + | | OCMIP |
| LOVECLIM | ECBILT | CLIO | CLIO | | + |
| McGill Paleoclimate model-2 | + | + | + | + | |
| MIT IGSM | GISS-GCM | + | - | | |
| MoBidiC | + | + | + | + | + |
| Planet Simulator | PUMA | + | + | | |
| Uvic | EMBM | GFDL | + | MOSES2 | NPZD |

***Table 8.*** *Details of EMIC models (continued)*

| EMIC Model | Terrestrial biosphere | Ice sheets | Interactive carbon cycle | Miscellaneous |
|---|---|---|---|---|
| Bern 2.5D | + | - | - | Season by season cycle |
| Climber-2 | VECODE | SICOPOLIS | + | |
| Climber 3α | VECODE | - | + | |
| GENIE | TRIFFID | GLIMMER | + | Ocean sediment (SEDGEM) |
| IAP RAS | + | + | - | |
| ISAM-2 | + | | + | Biomass burning, biogen emission |
| LOVECLIM | VECODE | + | VECODE.LOCH | |
| McGill Paleoclimate model-2 | VECODE | + | | |
| MIT IGSM | CLM | - | | |
| MoBidiC | VECODE | + | + | |
| Planet Simulator | + | | | It is appropriate for other planets and moons |
| Uvic | TRIFFID | UBC | | |

*Regional modelling of climate*

The method of the regional modelling of climate is the downscalling of the global models. There are some ways to do this:

- Statistical downscalling : There is a connection between the characteristics of global and regional models on the basis of historical behaviour. (Like generally in Hungary (*Tóth, 2005*).)
- Dynamical procedure: application of high resolution climate models
- Application of different resolution climate models
- Application of limited range climate models


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## Biotic feedbacks

Anthropogenic warming, rising of the sea-level could continue for centuries due to the scale of climate processes and feedbacks depending on whether the concentration of green house gases are managed to be stabilized (*Meehl et al., 2007; Denman et al., 2007*). The feedback between climate and carbon cycle gets surplus carbon dioxide to the atmosphere while the climate system is warming. The strength of this feedback is uncertain.

There are two types of natural ecosystems regarding to their place: terrestrial and oceanic ecosystems. Considering the biotic feedbacks larger order of magnitude $CO_2$ gets back to the atmosphere by terrestrial ecosystems.

### *Biotic feedbacks of oceanic ecosystems*

The main element of the oceanic ecosystems and biogeochemical cycle is the carbon. One of the most important feedback loops of oceanic carbon cycle and climate is the following: the increasing atmospheric $CO_2$ leads to increasing radiative forcing which results higher sea surface temperature (SST) and lower salt-content of sea surface water layers due to stronger hydrological cycle. Therefore it could induce the transformation of the thermohaline circulation in North Atlantic Deep Water, as well as modification of the oceanic carbon cycle, and then the decrease of the transport of the anthropogenic carbon from sea surface into the depth. The decreasing oceanic $CO_2$ uptake could accelerate the increase of atmospheric carbon dioxide.

Other biotic feedback is the production of dimethyl sulphide by phytoplankton. Dimethyl sulphide oxidizes in the atmosphere and could form aerosols which decrease the global warming (*Simo, 2001*).

Thirdly, the work of the oceanic organic carbon pump is limited by the amount of mineral resources. Iron and other materials get into the ocean by wind and have a great effect on bond of nitrogen and the amount of the oceanic primer production (*Falkowski et al., 1998*). Therefore the oceanic net primer production does not increase with the increasing atmospheric $CO_2$ (*Zondervan, 2007*).

### *Biotic feedbacks of terrestrial ecosystems*

*Charney et al., 1975* were the first who examined the effect of ecosystems to the climate. The examination was about feedbacks caused by changes of the surface of the Sahara. Later the global climate models consist of complex effects of land surface and atmosphere. Nowadays it has become important to investigate the effect of the vegetation of terrestrial ecosystems for the climate (*Friedlingstein et al., 2006; Meir et al., 2006*). As the role of the terrestrial ecology increases in Earth system modelling (EMIC) the feedbacks of the vegetation and soil processes for the climate are determined, and the effect of the land-use change to carbon cycle becomes more clear.

Biotic feedbacks of terrestrial ecosystems to climate are made by various biochemical circles. The global carbon cycle has the largest quantities of fluxes as it can be seen in *Table 9*.


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***Table 9.*** *Natural and anthropogenic quantities of chemical element cycles (Falkowski et al., 2000)*

| Element | Flux | Natural (*1000kg) | Anthropogenic (*1000kg) |
|---|---|---|---|
| C | Terrestrial respiration and demolition | 61000 | |
| | Fossil fuel and land use change | | 8000 |
| N | Natural biological bonding | 130 | |
| | Bonding in rice cultures, fertilization and burning of fuels | | 140 |
| P | Chemical crumbling | 3 | |
| | Mining | | 12 |
| S | Natural emissions | 80 | |
| | Burning of fossil fuels and biomass | | 90 |
| O and H (water) | Precipitation | $111*10^{12}$ | |
| | Global water using | | $18*10^{12}$ |

Three- or four- fold carbon is stored in terrestrial ecosystems than in the atmosphere and more than one eighth of the atmospheric carbon dioxide passes through the ecosystems in a year due to photosynthesis and respiration. Processes of carbon cycle take a feedback to climate which can interpret on two levels: top-down, approaching from the whole biosphere and bottom-up, building from the fundamental biogeochemical processes (see Table 3).

The main feedbacks of carbon cycle and climate are (*Luo et al., 2001*):

- a positive feedback through photosynthesis, vegetation growth and respiration
- a negative feedback through photosynthesis, vegetation and carbon sequestration
- acclimatization of soil respiration to warming weakens the positive feedback

Present day status of the global carbon cycle is important to focus to the following researches (*Canadell et al., 2004*):

- Determination of forests' biomass
- Eddy covariance flux net
- Determination of carbon content of soil
- Mineral materials' transport from rivers to seas
- Measuring the emission of fossil fuels
- N, P, Si, Fe-fluxes to ecosystems
- Non-$CO_2$ emission of ecosystems (CO, $CH_4$, VOC)
- Atmospheric $CO_2$ in space

If these values are available we can size up more precisely the carbon sources and sinks.

*Possible biotic feedbacks*

$CO_2$ and $CH_4$ are the essential parts of carbon cycle. They make an important role in regulating of ecosystems. Climate could also be affected by biogen aerosols (e.g. isoprene).


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- Equilibrium of photosynthesis and respiration
  Increasing atmospheric $CO_2$ ($CO_2$-fertilization) gives negative feedback to climate because plants could take up more carbon which decreases the amount of the carbon dioxide in the atmosphere.
  There are many experiments to estimate the effect of the fertilization (CFE). *Lobell and Field, 2008* have found, that an average effect of a 1 ppm increase of $CO_2$ on yields of $C_3$ plants (rice, wheat, maize) is 0.1%. So for an average year 0.14% yield increase is available (with 0.07% dispersion).
- Methane emission of wetlands
  There are three important kinds of control for the methane emission: temperature of the soil, depth of water table, size of the opened soil layer. Methane could flow to the atmosphere in several ways, such as with molecular diffusion, bubbling up or through stem of vessel plants. Methane is a green house gas, so reaching the atmosphere the temperature will increase, which results n more methane emissions.
  Wetlands and flooded lands (considering the rice lands) spread in $8.6 \cdot 10^6$ $km^2$ area, an amount of $4.6 \cdot 10^6$ $km^2$ of which are in tropical and subtropical location (*Clarke, 1994*). Methane emission is 115 – 237 Tg $CH_4$/year (*Gedney et al., 2004*).
- Biogen aerosols
  Aerosols play an important role in climate system, they absorb, reflect or scatter the incoming solar radiation, so they have a cooling effect. The distribution of several aerosols can be found in *Table 10*. Many volatile organic compounds get to the atmosphere which gives secondary organic aerosols reacting with hydroxyl residue. There are two classes of SOAs regarding to their genesis: biogen SOA (90%) formed by oxidation of volatile organic compound (VOC) and anthropogenic SOA (10%) formed by oxidation of anthropogenic VOCs. Forests are great isoprene-emitters; the emission can be even 300-500 Mt C/year.

***Table 10.*** *Distribution of aerosols (Kanakidou et al., 2005)*

| Source | Total amount (Tg/year) |
|---|---|
| Biomass burning | 54 (45-80) |
| Fossil fuels | 28 (10-30) |
| Biogen secondary organic aerosol | 16 (8-40) |
| Anthropogenic secondary organic aerosol | 0.6 (0.3-1.8) |
| Sum of organic substance | 98 (60-150) |
| Sum of aerosols | 800 |

- Soil respiration
  Respiration of soil accelerates with the increase of the temperature, so more $CO_2$ goes to the atmosphere which strengthens the global warming. *Raich et al., 2005* examined the amount of the $CO_2$ flux between soil and atmosphere between 1980 and 1994. They have found that the value of the average flux was 80.4 Pg C (79.3-81.8 Pg C) annually; and considering the changes of temperature during a year they have measured 3.3 PgC/year/°C.
- More $CO_2$ content of soil and fallen leaves (*Kimball et al., 2001*)


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Less nitrogen admittance is caused by increased atmospheric carbon dioxide concentration indirectly which decelerates the growth of the plants.

- Effect of warming to plants (*Feeley et al., 2007*)
  More water use is caused by warming, the $CO_2$ uptake and photosynthesis rate is decreased by shift of the temperature from optimum which is originated from less conductivity of stomas. On the other hand, the density and the number of stomas increase on higher temperature and $CO_2$ concentration (*Pandeya et al., 2007*).
- Fire frequency (*Running, 2006*)
  Occurance of fire becomes more frequent with warming decreasing the carbon content of the terrestrial ecosystems and increasing the concentration of $CO_2$ in the air which increases the temperature.

*Coupled carbon cycle-climate models*

Feedback of the global carbon cycle is investigated with the help of GCM and EMIC modelling. There are some studies that consider the oceanic carbon cycle's feedbacks (*Joos et al., 1999*), only, while EMIC model examines both the oceanic and the terrestrial connections. At first the feedbacks of terrestrial and oceanic ecosystems to climate were simulated by *Cox et al., 2000,* Hadley Centre, Great Britain. A three-dimensional model has been developed to show, that feedbacks of the global carbon cycle could accelerate the global warming during the 21st century significantly.

During modelling HadCM3 climate model connected with an oceanic carbon cycle model (HadOCC) and a terrestrial dynamic vegetation model (TRIFFID) was used. Three different runs were made to isolate the effect of climate and the feedback:

- IS92a $CO_2$ emission and fixed vegetation (standard)
- Interactive $CO_2$ emission and dynamic vegetation, supposed that $CO_2$ does not affect the climate (off-line)
- Fully coupled climate-carbon cycle model simulation

The results show that terrestrial carbon sequestration could decrease with global warming; especially in those regions where the increase of the temperature is not advantageous regarding the photosynthesis. In case of low $CO_2$ concentration the direct effect of $CO_2$ dominates and the carbon content of soil and vegetation increases with atmospheric carbon dioxide. Moreover, the increase of the $CO_2$ concentration the carbon content of terrestrial ecosystems begins to decrease because of the respiration of the soil. The intermediate term between the two systems will be about 2050. The $CO_2$ concentration is going to be 980 ppm by 2100 considering the positive feedback of the carbon cycle to the climate.

$CO_2$ uptake of the ocean decreases during the years, the rate will be 5 GtC/year. The different temperatures (due to the increase of the sea surface temperature) hinder to evolve the $CO_2$ gas which decreases the obtainability of nutrient and the net primer production (with 5%).

*Dufrense et al., 2002* have made similar simulations in IPSL, France. They have compared the data from Hadley Centre (*Friedlingstein et al., 2003*). The studies agree in the positive feedback of carbon cycle to climate, but the rate of this feedback is different. They agree that $CO_2$ uptake of the ocean would hardly change during the next century. This is in contrast with the previous, only-ocean models according to which the ocean carbon uptake is decreased by climate change (*Joos et al., 1999; Sarmiento et al., 1998*).


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According to *Zeng et al., 2004*, Maryland University, USA, the difference between the outputs of the simulations is caused by the determination of the terrestrial carbon content.

The model approach has been improved by C. Jones and his collaborates to reply the results from the Hadley Centre (*Jones et al., 2003*). The previous used climate model was completed with an interactive carbon cycle considering the cooling effect of the sulphate aerosols, so the overestimation of the $CO_2$ concentration (*Cox et al., 2000*) was disappeared.

There is a positive feedback of the terrestrial ecosystems to climate but their size is quite uncertain (*Govindasamy et al., 2004; Joos et al., 2001*). There were investigations to the future in the frame of $C^4$MIP (Coupled Climate-Carbon Cycle Model Intercomparison Project) (*Friedlingstein et al., 2006*). The model consists of seven coupled ocean-atmosphere general circulation models (OAGCM) and four Earth System models of intermediate complexity (EMIC). These simulations ran for the past and for the 21st century. Every model had the same $CO_2$ emission values for the past and for the future (IPCC SRES A2 scenario).

Most of the models contain the $CO_2$ emissions related to the land-use change but none of the models use the actual Earth surface changes. Therefore the related physical and biogeochemical processes were ignored during the studies.

Two simulations were run with every model: the first is the coupled case where the climate-change affects the carbon cycle and the second one is the non-coupled case where the $CO_2$ was a non-radiant active gas. The difference between the two cases specifies the effect of the climate to the global carbon cycle.

The differences between the various coupled models with the increase of the $CO_2$ concentration will become important by about 2025. Comparing the coupled cases to uncoupled ones the $CO_2$ concentration becomes higher in the former cases. Every model has positive climate-carbon cycle feedback. The amount of this surplus $CO_2$ is quite uncertain; its value will be between 20 ppm and 220 ppm by 2100.

A feedback analysis was performed to $CO_2$ concentration. The effect of the $CO_2$ change to the global average temperature is as follows:

$$\Delta T^c = \alpha \Delta C_A^c \quad \text{(Eq.5)}$$

where $\Delta T^c$ is the increasing temperature (K), $\Delta C_A^c$ the atmospheric $CO_2$ concentration (ppm), $\alpha$ is the linear transient climate sensitivity, $c$ is related to the coupled model and $u$ is related to the uncoupled case.

The additional warming due to the climate-carbon cycle feedback is:

$$\Delta T^c - \Delta T^u = \alpha\left(\Delta C_A^c - \Delta C_A^u\right) \quad \text{(Eq.6)}$$

So there is an additional 0.1-1.5$^0$C warming by 2100 due to the feedback effect.

The positive value of the climate-carbon cycle feedback means that the amount of the permissible emissions should be decreased (*Jones et al., 2006*).

## Conclusion

The environment, the local and the global climate are affected by the ecosystems through the climate-ecosystem feedbacks. There is a great amount of carbon in the living vegetation and the soil like organic substance which could be formed to atmospheric $CO_2$ or methane hereby affecting the climate. $CO_2$ is taken up by terrestrial ecosystems during the photosynthesis and is lost during the respiration process, but


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carbon could be emitted like methane, volatile organic compound and solved carbon. The feedback of the climate-carbon cycle is difficult to determine because of the difficulties of the biological processes.

The biological simplification is essential during the modelling of vegetation processes. It is important to consider more feedbacks to the climate system to decrease the uncertainty of the estimations.

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# THE BEGINNING AND THE END OF THE INSECTS' FLIGHT TOWARDS THE LIGHT ACCORDING TO DIFFERENT ENVIRONMENTAL LIGHTINGS

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**Abstract.** Many news bulletins found in the literature only consent themselves with the description of the night distribution of the trapped species not examining the beginning and the end of the insects' flight towards the light with the measurement of environmental lighting, expressed in lux. For this reason, we had examined the daily appearance of the first and last specimens of the species in the light trap concerning the exact lighting figures. We have used the hourly collection data of the fractionating light trap at the farm of Julianna in Nagykovácsi, belonging to the MTA Crop Protection Research Institute. With the help of our own computer programme we had counted the light coming from the sun, moon and from the starry sky, for every full hour separately and in total. We had given two lighting figures to every trapping data: we had counted the first minute of the first and the next hour of the given hour in lux within which the trapping had happened. Hereby, two lighting figures had become known during which the flight towards lighting had begun and end. The flight of 51 species towards the light happens when the total of the given hour concur in the duration of navigation twilight, of 26 species in the duration of sidereal twilight and of 7 species in the duration of night light. There were 2 species where the first imago was already captured during daylight. In the period of the quick reduction of lighting only the first specimens of the 14 species appear, accordingly: 4 from daylight to civil twilight, 4 from daylight to navigation twilight, 1 from sunset to navigation twilight and 4 species from civil twilight to sidereal twilight. The flight towards light ends in the case of 16 species after midnight during the night light, at 48 species during sidereal twilight and at 28 species at navigation twilight. The flight of only 3 species end when it is clearing up quicker within the given hour, from navigation twilight to daylight. Our results could stop a gap.
**Keywords:** *Lepidoptera, flying threshold, lux*

## Introduction and survey of literature

The majority of researchers do not examine the beginning and the end of the insects' flight towards the light with the measurement of environmental lighting, expressed in lux. Most of the authors were satisfied to describe the night distribution of the trapped insects. We only sporadically find records in the literature in reference to at how much lux lighting we can experience the start of certain insects' flight.

According to Mazorchin-Porsnjakov [1] the species of the chestnut cockchafer (Melolontha hippocastani Fabr.) start flying at 14 lux but can only be collected with light trap when lighting is decreased to 7-8 lux.

Tshernyshev [2] had carried out light-trap collectings and visual observations around Moscow. In reference to many insect classes and many important species, he also


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reported the associated measurement of lighting in lux to the start and maximum of activity.

- Ephemeroptera: *Ordella horaria* L. 4-0.005 lux;
- Homoptera: *Psylla betulae* L. 0.001 lux;
- Heteroptera: *Sigara falleni* Fieb.. *S. striata* L.. *S. praeusta* Fieb. 10 lux;
- Coleoptera: *Amara majuscula* Chd. 1-0.5 lux, *Hybius ater* DeGeer 0.01 lux, *Hydrobius fuscipes* L. 1 lux, *Cercyon quisquillus* L., *C. haemorrhoidalis* Fabr., *C. unipunctatus* L., *C. melanocephalus* L. 5-1 lux, *Serica brunnea* L. 0.001 lux, *Necrophorus vespillo* L. 10-0.1 lux, *Oxytellus rugosus* L. 1 lux, *Bledius opacus* Block. and *Heterocerus hispidulus* Kieff. 100-10 lux, *Aphodius rufus* Müll. *A. distinctus* Müll. 10-1 lux, but these last mentioned species had flew at 10.000 lux as well in May, daytime at 15-17 degrees Celsius.
- Hymenoptera: *Ophion luteus* L. 0.01 lux, *Lasius niger* L. 1000-50 lux;
- Diptera: *Culicoides pulicaris* L. and *C. grisescens* Edw. 10-0.03 lux;
- Trichoptera: *Psychomya pusilla* Fbr., *Leptocerus dissimilis* Steph., *L. nigriversosus* Retz., *Hydropsyche ornatula* McLeach., *Halesus interpunctatus* Zett. 0.1-0.01 lux;
- Lepidoptera: most of the species between 0.01-0.001 lux, but *Hepialus sylvinus* L., H. humuli L. 5-1 lux.

Skuhravý and Zumr [3] had studied the night activities of the black-arches moth (Lymantria monacha L.) in pine tree stand in the Czech Republic. Their flight towards light had began when lighting decreased to 1-3 lux. Through Dreisig's [4] studies in Denmark and Florida he had allocated that the beginning of activities of certain species are specific, usually starts between 1 and 0,003 lux at invariant lighting. If the period of twilight increases, the dispersion of the invariant rate will be higher. This is also influenced by the season besides the geographical latitude. He also gives data about the beginning of the flight of the Macrolepidoptera species related to the environmental lighting. These are the following in Denmark: Plusia gamma L. 1-12 lux, *Agrotis exclamationis* L. 0.4 lux, *Caradrina morpheus* Hfn. 0.15 lux, *Typhena pronuba* L. 0.9 lux, *Monima pulverulenta* Esp. 0.09 lux, *Cerapteryx graminis* L. 0.8 lux, *Deilephia porcellus* L. 1.4 lux, *Malacosoma castrensis* L. 0.5 lux. In Florida: *Plusia gamma* L. 0.8 lux, *Heliothis virescens* Fabr. 0.03 lux, *Spodoptera frugiperda* Smith 0.007 lux, *Anticarsia gemmalis* Hbn. 0.08 lux, *Mocis latipes* Guenee 0.009 lux, *Schinia nubila* Str. 0.15 lux, *Megalopyge opercularis* Abbot & Smith 0.02 lux, *Nystalea* sp. 0.004 lux.

As there are not too many data in literature about the beginning and the end of flight towards the environmental light that is given is lux, we have examined the day-by-day appearance of the first and last specimens of certain species using our domestically collected figures.

## Materials

At the Crop Protection Research Institute near Budapest, we had operated a fractionating light trap between 1976-1979 at the research plant of Julianna, in Nagykovácsi and the insects collected there had been separated into different flasks. The light trap had operated with a 125-watt HGL bulb. However, this light trap did not operate every night, only periodically, 57 times altogether but that time for 12 hours, in the spring and summer time, early in the autumn from 5 p.m. to 5 a.m. and in the second


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half of October from 4 p.m. to 4 a.m. (UT). These years the daylight saving time was not applied. Mészáros had defined and journalized from the collected insects the insect pests from all Macrolepidoptera and Microlepidoptera species. We had used the records of 160 Lepidoptera- and 1 Coleoptera species for our work.

The operation had taken part on the following days:

1976: 08. 26-27, 10. 06-07;

1977. 03.10-11, 03.14-15, 03.16-17, 03.17-18, 03.18-19, 03.19-20, 03.21-22, 03.22-23, 03.24-25, 03.28-29, 04.14-15, 04.18-19, 04.19-20, 04.20-21, 04.22-23, 04.26-27, 04.28-29, 05.04-05, 05.09-10, 05.12-13, 05.16-17, 05.19-20, 05.28-29, 05.30-31, 06.02-03, 06.06-07, 06.09-10, 06.13-14, 06.16-17, 06.21-22, 06.24-25, 06.28-29, 07.04-05, 07.12-13, 07.26-27, 07.27-28, 08.08-09, 09.06-07, 09.15-16, 09.22-23, 10.06-07, 10.13-14, 10.20-21, 10.2-28

1978. 09.18-19, 09.26-27, 11.02-03

1979. 03.22-23, 03.28-29, 05.10-11, 05.17-18, 05.25-26, 06.20-21, 06.22-23, 07.23-24.

We had counted the lighting data required for our examinations with the help of our own computer programme. György Tóth, astronomer – who unfortunately cannot be with us any longer – for a TI 59, established this programme. Computer, to be used in our common researches (Nowinszky and Tóth, [5]). This programme was adapted to a modern computer by Miklós Kiss, associate professor, for which hereby we would like to express our thanks.

The programme counts the daytime and twilight lighting from the sun to optional geographical place, day and time separately and in total, the light of the moon if it is over the horizon and the lighting coming from the starry sky, all these data in lux. It also takes the number of clouds into consideration when counting.

We had collected every data concerning all clouds from the yearbooks of the National Meteorological Organization. In these, data is recorded every 3 hours with causal explanation. We had applied the data to the related given hour and to the next 2 hours.

## Methods

We have collected the hour of the capture of the first and last specimens of the trapped species from the light trap journal in reference to every night. We have counted the full lighting figures of these time periods. As, of course, the exact trapping time is unknown the lighting figures were counted in reference to a whole hour. We had given two lighting figures to every trapping data: we had counted the first minute of the first and the next hour of the given hour in lux, within which the trapping had happened. Hereby, two lighting figures had become known during which the flight towards lighting had begun and end.

By species, we had put the lux value pairs into order according to the first and last figures of trapping. Our figures were put into a table. We had placed those lighting value pairs into this table, in which the first specimen is already, the last still flew, also separated accordingly whether the trapping had happened before or after midnight.


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## Results

*Table* 1. contains the figures of 161 species. We had also aspired to include as many information as possible. To achieve this we had also given to every lux value the period of twilight or night, it belonged to. The abbreviations of these are the following: the numbers in italics show the trappings after midnight, * = only one figure apply to a given specimen, T/N = twilight or night hour, D = daylight, S = sunset, C = civil twilight, A = sidereal twilight, NS = the light of the night sky

## Discussion

The beginning of flight towards the light at night happens at distinct lighting conditions in the case of certain species. These do not indicate lawfulness that should be linked to taxonomical rating. The flight of 50 species towards the light happens when the total of the given hour concur in the duration of navigation twilight, of 26 species in the duration of sidereal twilight and of 7 species in the duration of night light. There were 2 species where the first imago was already captured during daylight. In the period of the quick reduction of lighting only the first specimens of the 14 species appear, accordingly: 4 from daylight to civil twilight, 4 from daylight to navigation twilight, 1 from sunset to navigation twilight and 4 species from civil twilight to sidereal twilight.

The flight towards light ends in the case of 16 species after midnight during the night light, at 48 species during sidereal twilight and at 28 species at navigation twilight. The flight of only 3 species end when it is clearing up quicker within the given hour, from navigation twilight to daylight.

Although we only have a few results, many of these are often from one collection figure, we believe it is worth to share with our readers. On the one hand because we could not find any researches like this in the literature, which publish the flight peculiarities of so many species, on the other hand because those are also not from mass collection figures. Of course, our published results will be altered by our continuous observations but until then with their informative nature can stop a gap. We could get interesting informations for example from entomologists who should journalize the exact arrival time of the insects into the capturing sheet and should also measure the lighting related to it. The measuring instrument needed for this is fairly easy to access nowadays and although they are not occupied with, researches like this could help the entomological studies with very important and precise data.


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***Table 1.*** *Beginning and ending of flight of Lepidoptera species before and after midnight in connection with the twilights*

| ***Species*** | ***Beginning and ending of flight (before midnight)*** | | | | ***Ending of flight (before or after midnight)*** | | | |
|---|---|---|---|---|---|---|---|---|
| | ***between*** | | | | ***between*** | | | |
| ***Lepidoptera*** | ***Lux*** | ***T/N*** | ***Lux*** | ***T/N*** | ***Lux*** | ***T/N*** | ***Lux*** | ***T/N*** |
| *Plutellidae* | | | | | | | | |
| Plutella maculipennis Curt. | 79.22 | C | 0.1450 | N | 0.0019 | A | 0.0071 | A |
| *Gelechiidae* | | | | | | | | |
| Anarsia lineatella Zeller * | 0.0384 | N | 0.0388 | N | | | | |
| Recurvaria leucatella Clerck. | 0.0028 | A | 0.0031 | A | *0.0987* | *N* | *1.821* | *C* |
| Recurvaria nanella Hbn. | 0.0388 | N | 0.0391 | N | 0.0039 | A | 0.0029 | A |
| Sitochroga verticalis L. | 0.1450 | N | 0.1003 | N | *0.0203* | *N* | *1.999* | *N* |
| *Tortricidae* | | | | | | | | |
| Pandemis heparana Schiff. | 1386.2 | D | 30.338 | C | 0.0017 | A | 0.0017 | A |
| Pandemis ribeana Hbn. | 1386.20 | D | 41.614 | C | 0.0717 | N | 0.0714 | N |
| Argyrotaenia pulchellana Haw. * | | | | | *0.0396* | *N* | *0.0472* | *N* |
| Adoxophyes reticulana Hbn. | 0.0388 | N | 0.0391 | N | *0.0039* | *A* | *1.001* | *N* |
| Hedya nubiferana Haw. | 649.680 | D | 2.5273 | N | *0.0422* | *N* | *90.57* | *C* |
| Spilonota ocellana F. | 12.6161 | C | 0.0114 | N | *0.0019* | *A* | *0.0071* | *A* |
| Cydia pomonella L. | 737.302 | D | 0.3745 | N | *0.0276* | *N* | *186.47* | *C* |
| Tortrix viridana L. * | 0.0604 | N | 0.0216 | N | | | | |
| *Phycitidae* | | | | | | | | |
| Oncocera semirubella Scop. | 0.0495 | N | 0.0453 | N | *0.0012* | *A* | *0.0039* | *A* |
| Etiella zinckenella Tr. | 0.0195 | N | 0.0178 | N | 0.0022 | A | 0.0031 | A |
| *Pyraustidae* | | | | | | | | |
| Ostrinia nubilalis Hbn. | 0.0021 | A | 0.0021 | A | *0.0045* | *A* | *0.0067* | *A* |
| Loxostege sticticalis L. * | 0.0029 | A | 0.0028 | A | | | | |
| Evergestis extimalis Scop. | 0.0708 | N | 0.0717 | N | *0.0104* | *N* | *0.0086* | *A* |
| Evergestis frumentalis L. | 0.0960 | N | 0.0495 | N | 0.0195 | N | 0.0178 | N |
| *Geometridae* | | | | | | | | |
| Alsophila aescularia Schiff. | 346.480 | C | 0.0451 | N | 0.0005 | NS | 0.0005 | NS |
| Aplocera plagiata L. | 0.0101 | A | 0.0094 | A | *0.0012* | *A* | *0.0012* | *A* |
| Operophthera brumata L. | 0.0014 | A | 0.0014 | A | *0.0368* | *N* | *0.0357* | *N* |
| Philereme vetulata Schiff. | 30.3375 | C | 0.0279 | N | 0.0363 | N | 0.0165 | N |
| Lygris pyraliata Schiff. | 0.0495 | N | 0.0453 | N | 0.0111 | N | 0.0111 | N |
| Cidaria fulvata L. | 0.0165 | N | 0.0111 | N | 0.0021 | A | 0.0021 | A |
| Xanthorrhoe fluctuata L. * | 0.0008 | NS | 0.0004 | NS | | | | |
| Hydrelia flammeolaria Hfn. * | 0.0025 | A | 0.0021 | A | | | | |
| Eupithecia centaureata Schiff. * | 0.0717 | N | 0.0714 | N | | | | |
| Bapta temerata Schiff. * | 0.0034 | A | 0.0008 | NS | | | | |
| Ennomos erosaria Schiff. * | 0.1808 | N | 0.1385 | N | | | | |
| Colotois pennaria L. * | | | | | *0.0014* | *A* | *0.0012* | *A* |


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|---|---|---|---|---|---|---|---|---|
| | ***between*** | | | | ***between*** | | | |
| ***Lepidoptera*** | ***Lux*** | ***T/N*** | ***Lux*** | ***T/N*** | ***Lux*** | ***T/N*** | ***Lux*** | ***T/N*** |
| Crocallis elinguaria L. * | 0.0008 | NS | 0.0008 | NS | | | | |
| Plagodis dolabraria L. * | 0.0321 | N | 0.0346 | N | | | | |
| Macaria alternaria Hbn. * | 0.0178 | N | 0.0164 | N | | | | |
| Chiasmia clathrata L. | 0.8153 | N | 0.0004 | NS | *0.8153* | *N* | *611.76* | |
| Erannis leucophaearia Schiff. * | 0.0211 | N | 0.0026 | A | | | | |
| Erannis marginaria Bkh. | 0.0332 | N | 0.0238 | N | 0.0005 | NS | 0.0005 | NS |
| Apocheima hispidaria Schiff. | 346.480 | C | 0.0451 | N | 0.0006 | NS | 0.0006 | NS |
| Nyssia zonaria Schiff. | 346.480 | C | 0.0451 | N | *0.0010* | *A* | *0.0010* | *A* |
| Lycia hirtaria Cl. | 2853.20 | D | 17.124 | C | *0.0010* | *A* | *0.0010* | *A* |
| Biston stratarius Hfn. | 346.480 | C | 0.0451 | N | 0.0008 | NS | 0.0008 | NS |
| Biston betularius L. | 0.0631 | N | 0.0009 | NS | 0.0425 | N | 110.41 | C |
| Boarmia rhomboidaria Schiff. | 32.504 | C | 0.0311 | N | *0.0031* | *A* | *0.0048* | *A* |
| Boarmia cinctaria Schiff. * | 0.1389 | N | 0.1569 | N | | | | |
| Biston arenaria Hfn. | 0.0014 | A | 0.0006 | NS | 0.0007 | NS | 0.0007 | NS |
| Ascotis selenaria Schiff. * | 0.0128 | N | 0.0118 | N | | | | |
| Ectropis bistortata Goeze * | | | | | *0.0054* | *A* | *0.0063* | *A* |
| Ematurga atomaria L. * | 0.0363 | N | 0.0040 | A | | | | |
| Siona lineata L. | 0.0008 | NS | 0.0004 | NS | 0.0717 | N | 0.0714 | N |
| *Noctuidae* | | | | | | | | |
| Colocasia coryli L. * | | | | | *0.0840* | *N* | *0.0888* | *N* |
| Apatele rumicis L. | 0.5266 | N | 0.0086 | A | 0.0006 | NS | 0.0003 | NS |
| Euxoa temera Hb. * | 0.1258 | N | 0.0228 | A | | | | |
| Euxoa obelisca Schiff. | 0.0029 | A | 0.0028 | A | *0.0070* | *A* | *0.0055* | *A* |
| Agrotis ypsilon Rott. | 0.0960 | N | 0.0495 | N | 0.0015 | A | 0.0015 | A |
| Scotia segetum Schiff. | 32.5042 | C | 0.0311 | N | *0.0086* | *A* | *0.0088* | *A* |
| Scotia exclamationis L. | 2.5273 | N | 0.0034 | A | *0.0857* | *N* | *0.5864* | *N* |
| Eugnorisma depuncta L. * | 0.0134 | N | 0.0106 | N | | | | |
| Diarsia rubi View. * | 0.0604 | N | 0.0216 | N | | | | |
| Xestia c-nigrum L. | 46.006 | C | 0.0604 | N | 0.0007 | NS | 0.0007 | NS |
| Epipsilia grisescens F. * | | | | | *0.0004* | *NS* | *0.0008* | *NS* |
| Ochropleura plecta L. | 0.0063 | A | 0.0061 | A | 0.0013 | A | 0.0007 | NS |
| Diarsia rhomboidea Schiff. | 0.0028 | A | 0.0026 | A | 0.0009 | NS | 0.0009 | NS |
| Diarsia xanthographa Schiff. * | 0.0057 | A | 0.0045 | A | | | | |
| Cerastis rubricosa Schiff. | 17.1235 | C | 0.0049 | A | *0.0124* | *N* | *1.6583* | *N* |
| Ammoconia caecimacula Schiff. | 18.092 | C | 0.1921 | N | *0.0037* | *A* | *0.0029* | *A* |
| Noctua pronuba L. | 16.6122 | C | 0.0134 | N | 0.0009 | NS | 0.0012 | A |
| Triphaena orbona Hfn. | 0.0057 | A | 0.0045 | A | *0.0012* | *A* | *0.0012* | *A* |
| Mamestra brassicae Hfn. | 0.0142 | N | 0.0128 | N | 0.0007 | NS | 0.0007 | NS |
| Mamestra suasa Schiff. | 0.0128 | N | 0.0118 | N | *0.0054* | *A* | *0.0063* | *A* |


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|---|---|---|---|---|---|---|---|---|
| | ***between*** | | | | ***between*** | | | |
| ***Lepidoptera*** | ***Lux*** | ***T/N*** | ***Lux*** | ***T/N*** | ***Lux*** | ***T/N*** | ***Lux*** | ***T/N*** |
| Discestra trifolii Hfn. | 0.0279 | N | 0.0025 | A | 0.0118 | N | 0.0104 | N |
| Polia contigua Schiff. * | 0.0021 | A | 0.0021 | A | | | | |
| Harmodia luteago Schiff. | 0.0484 | N | 0.0039 | A | 0.0216 | N | 0.0195 | N |
| Tholera decimalis Poda * | | | | | *0.0019* | *A* | *0.0012* | *A* |
| Aplecta advena Schiff. | 0.0054 | A | 0.0045 | A | *0.0019* | *A* | *0.0071* | *A* |
| Xylomania conspicillaris L. | 0.1693 | N | 0.0912 | N | *0.0224* | *N* | *0.0257* | N |
| Perigrapha i-cinctum Schiff. | 221.151 | C | 0.0211 | N | 0.0089 | A | 0.0088 | A |
| Orthosia incerta Hfn. | 8507.98 | D | 676.8 | D | *0.0010* | *A* | *0.0010* | *A* |
| Orthosia gothica L. | 206.548 | C | 0.0163 | N | *0.0010* | *A* | *0.0010* | *A* |
| Orthosia munda Schiff. | 206.548 | C | 0.0163 | N | 0.0018 | A | 0.0019 | A |
| Orthosia stabilis Schiff. | 2853.20 | D | 17.124 | C | 0.0207 | N | 0.0209 | N |
| Orthosia miniosa F. * | 0.0005 | NS | 0.0005 | NS | | | | |
| Orthosia cruda Schiff. | 206.548 | C | 0.0163 | N | *0.0084* | *A* | *0.0083* | *A* |
| Mythimna ferrago F. * | 0.0279 | N | 0.0008 | NS | | | | |
| Mythimna albipuncta Schiff. | 0.1450 | N | 0.1003 | N | 0.0072 | A | 0.0080 | A |
| Mythimna l-album Esp. | *0.0070* | *A* | *0.0055* | *A* | *0.0041* | *A* | *0.0062* | *A* |
| Mythimna pallens L. | 0.3745 | N | 0.0126 | N | 0.0021 | A | 0.0021 | A |
| Cucullia argentea Hfn. | 0.5654 | N | 0.0014 | A | 0.3745 | N | 0.0126 | N |
| Phlogophora meticulosa L. * | 0.0065 | A | 0.0065 | A | | | | |
| Omphalophana antirrhini Hbn. * | 0.0604 | N | 0.0216 | N | | | | |
| Calophasia lunula Hfn. * | 0.0717 | N | 0.0717 | N | | | | |
| Brachinochia sphinx Hfn. | 2.5005 | N | 0.0024 | A | *0.0016* | *A* | *0.0016* | *A* |
| Lithophane ornitopus Hfn. | 221.151 | C | 0.0211 | N | *0.0010* | *A* | *0.0010* | *A* |
| Meganephria oxyacanthe L. * | 0.0015 | A | 0.0015 | A | | | | |
| Valeria oleagina Schiff. | 113.473 | C | 0.0139 | N | *0.0017* | *A* | *0.1383* | *N* |
| Crino satura Schiff. * | 16.6122 | C | 0.0134 | N | | | | |
| Agriopis convergens F. * | 0.0075 | A | 0.0046 | A | | | | |
| Drybotodes protea Bkh. | 0.0075 | A | 0.0046 | A | *0.0302* | *N* | *0.0900* | *N* |
| Antitype nigrocincta Tr. * | 0.1258 | N | 0.0228 | A | | | | |
| Eupsilia transversa Hfn. | 0.0228 | N | 0.0234 | N | *0.0014* | *A* | *0.0012* | *A* |
| Eupsilia satellitia L. | 60.7056 | C | 0.0073 | A | *0.0014* | *A* | *0.0014* | *A* |
| Conistra erythrocephala F. | 0.0234 | N | 0.0292 | N | 0.0005 | NS | 0.0005 | NS |
| Conistra vau-punctatum Esp. | 0.0013 | A | 0.0014 | A | *0.0087* | *A* | *0.0089* | *A* |
| Conistra vaccinii L. | 8507.98 | D | 676.80 | D | 0.0014 | A | 0.0014 | A |
| Agrochola humilis Schiff. | 0.3661 | N | 0.0083 | A | 0.0234 | N | 0.0260 | N |
| Agrochola lychnidis Schiff. * | 0.0134 | N | 0.0106 | N | | | | |
| Agrochola macilenta Hbn. | 0.0228 | N | 0.0234 | N | 0.0024 | A | 0.0014 | A |
| Agrochola helvola L. * | 100.105 | C | 0.0369 | N | | | | |
| Agrochola litura L. | 0.0369 | N | 0.0292 | N | 0.0105 | N | 0.0101 | N |


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| | ***between*** | | | | ***between*** | | | |
| ***Lepidoptera*** | ***Lux*** | ***T/N*** | ***Lux*** | ***T/N*** | ***Lux*** | ***T/N*** | ***Lux*** | ***T/N*** |
| Cosmia aurago F. * | 0.0165 | N | 0.0017 | A | | | | |
| Amphipyra pyramidea L. * | | | | | *0.0076* | *A* | *0.0276* | *N* |
| Procus strigilis Cl. | 0.0960 | N | 0.0495 | N | 0.0384 | N | 0.0388 | N |
| Luperina testacea Schiff. | 0.0094 | A | 0.0083 | A | 0.0057 | A | 0.0045 | A |
| Charanyca trigrammica Hfn. | 0.0976 | N | 0.0178 | N | 0.0029 | A | 0.0028 | A |
| Cosmia trapezina L. | 0.0128 | N | 0.0118 | N | 0.0021 | A | 0.0021 | A |
| Apamea anceps Schiff. | 164.304 | C | 0.0738 | N | *0.0203* | *N* | *1.9993* | *N* |
| Dicycla oo L. | 0.0165 | N | 0.0111 | N | 0.0021 | A | 0.0021 | A |
| Heliothis maritima Grasl. * | 0.0008 | NS | 0.0009 | NS | | | | |
| Chariclea delphinii L. * | 0.0484 | N | 0.0039 | A | | | | |
| Lithacodia deceptoria Scop. * | | | | | *0.0229* | *N* | *0.0468* | *N* |
| Erastria trabealis Scop. | 649.68 | D | 2.5273 | N | 0.0495 | N | 0.0453 | N |
| Tarache luctuosa Esp. | 0.0074 | A | 0.0057 | A | 0.0018 | A | 0.0012 | A |
| Hylophila prasinana L. * | 0.0008 | NS | 0.0009 | NS | | | | |
| Minucia lunaris Schiff. | 0.0738 | N | 0.0065 | A | *0.0381* | *N* | *0.1394* | *N* |
| Plusia chrysitis L. | 0.0105 | N | 0.0101 | N | 0.0006 | NS | 0.0003 | NS |
| Abrostola trigemina Wern. * | | | | | *0.0207* | *N* | *0.0229* | *N* |
| Autographa gamma L. | 0.0801 | N | 0.0840 | N | *0.0086* | *A* | *0.0126* | *N* |
| Hadena confusa Hfn. * | 16.6122 | C | 0.0134 | N | | | | |
| Episema coeruleocephala L. | 18.0918 | C | 0.1921 | N | *0.0320* | *N* | *0.3020* | *N* |
| Toxocampa craccae F. * | 0.0057 | A | 0.0045 | A | | | | |
| *Lymantriidae* | | | | | | | | |
| Dasychira fascelina L. * | | | | | *0.0010* | *A* | *0.0026* | *A* |
| Dasyhira pudibunda L. | 0.0321 | N | 0.0346 | N | *0.0054* | *A* | *0.0063* | *A* |
| Lymantria dispar L. | 15.1900 | C | 0.0042 | A | *0.0048* | *A* | *0.3514* | *N* |
| *Arctiidae* | | | | | | | | |
| Gnophria rubricollis L. * | 0.0037 | A | 0.0006 | NS | | | | |
| Ocnogyna parasita Hbn. | 0.0017 | A | 0.0020 | A | *0.0005* | *NS* | *0.0005* | *NS* |
| Phragmatobia fuliginosa L. | 15.1900 | C | 0.0042 | A | 0.0090 | A | 0.0074 | A |
| Spilosoma menthastri Esp. | 0.0363 | N | 0.0165 | N | 0.0034 | A | 0.0008 | NS |
| Eucharia costa Esp. | 0.0072 | A | 0.0080 | A | *0.6542* | *N* | *650.07* | *D* |
| Hyphantria cunea Drury * | 0.0034 | A | 0.0008 | NS | | | | |
| Arctia villica L. | 0.0691 | N | 0.0681 | N | 0.0216 | N | 0.0195 | N |
| *Notodontidae* | | | | | | | | |
| Stauropus fagi L. * | 0.0021 | A | 0.0023 | A | | | | |
| Dicranura ulmi Schiff. | 0.5168 | N | 0.0016 | A | *0.0224* | *N* | *0.0257* | *N* |
| Drymonia querna Schiff. * | | | | | *0.0840* | *N* | *0.0888* | *N* |
| Drymonia chaonia Hbn. | 11.9237 | C | 0.0037 | A | 0.0028 | A | 0.0006 | NS |
| Pheosia tremula Clerck * | 0.0237 | N | 0.0229 | N | | | | |


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| | ***between*** | | | | ***between*** | | | |
| ***Lepidoptera*** | ***Lux*** | ***T/N*** | ***Lux*** | ***T/N*** | ***Lux*** | ***T/N*** | ***Lux*** | ***T/N*** |
| Notodonta phoebe Sieb. * | 0.0321 | N | 0.0346 | N | | | | |
| Ptilophora plumigera Esp. * | 0.0024 | A | 0.0014 | A | | | | |
| Phalera bucephala L. | *0.0008* | *NS* | *0.0422* | *N* | *0.8153* | *N* | *611.76* | *D* |
| *Sphingidae* | | | | | | | | |
| Marumba quercus Schiff. * | 0.0976 | N | 0.0950 | | | | | |
| Mimas tiliae L. | 1.3611 | C | 0.0295 | N | | | | |
| Celerio euphorbiae L. * | 0.0018 | A | 0.0012 | A | | | | |
| Deilephila elpenor L. * | 0.0003 | NS | 0.0004 | NS | | | | |
| Pergesa porcellus L. | 0.0476 | N | 0.0237 | N | 0.0012 | A | 0.0019 | A |
| *Thyatiridae* | | | | | | | | |
| Polyploca diluta F. | 0.0075 | A | 0.0046 | A | *0.0076* | *A* | *0.0276* | N |
| Polyploca flavicornis L. * | 0.0067 | A | 0.0006 | NS | | | | |
| Polyploca ridens Hbn. | 0.1693 | N | 0.0912 | N | 0.0371 | N | 0.0381 | *N* |
| *Drepanidae* | | | | | | | | |
| Cylix glaucata Scop. * | 0.0009 | NS | 0.0009 | NS | | | | |
| Drepana binaria Hfn. | 0.0295 | N | 0.0321 | N | 0.0010 | A | 0.0010 | A |
| Asphalia ruficollis Schiff. | 500.670 | S | 0.0652 | N | 0.0020 | A | 0.0140 | N |
| *Synthomidae* | | | | | | | | |
| Amata phegea L. * | 0.0165 | N | 0.0111 | N | | | | |
| Dysauxes ancilla L. * | | | | | *0.0012* | *A* | *0.0039* | *A* |
| *Coleoptera* | | | | | | | | |
| *Melolonthidae* | 676.8 | D | 0.4073 | N | | | | |
| Melolontha melolontha L. * | 676.8 | D | 0.4073 | N | | | | |

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