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Global Journal of Advanced Biotechnology & Biochemistry Research

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A BIOCHEMICAL STUDY ON ANTIOXIDANT ENZYMES IN CITRUS FRUITS PEEL

Mala Kumari

Abstract

Three species of citrus fruits were chosen to investigate and evaluate levels of enzymatic antioxidants in peels of C.reticulata, C.sinensis and C. aurentifolia. The results indicate that amongst these 3 citrus fruits peels; the specific activity (U mg^{-1}protein) of enzymatic antioxidants viz., ascorbic acid oxidase, guaiacol peroxidase, glutathione reductase, polyphenol oxidase and superoxide dismutase were highest in peels of C.aurentifolia (201 ± 18.05, 4.82 ± 1.62, 0.369 ± 0.03, 9538.4 ± 8.32 and 28.56 ± 2.90 respectively) with the exception of catalase which was highest in peels of C.sinensis (1310 ± 9.56 U mg^{-1}protein). The results obtained suggest metabolism of enzymatic antioxidants is more or less similar in citrus fruits peel indicate a potential for cheap and readily available natural source of antioxidants with health protective potential, which can be used in pharmaceutical, nutraceutical and food preparations.

Key words: Enzymatic antioxidants, Citrus peels, glutathione reductase, polyphenol oxidase, superoxide dismutase .

Introduction:

Citrus fruits have long been valued as part of a nutritious and tasty diet. The flavours provided by citrus are among the most preferred in the world and it is increasingly evident that citrus not only tastes good, but is also good for people. It is well established that citrus and citrus products are a rich source of vitamins, minerals and dietary fiber (non-starch polysaccharides) that are

essential for normal growth and development and overall nutritional well-being. **Manthley and Grohmann (2001) and Anagnostopoulou et al., (2006) c**itrus fruits are the world's most popular fruits. Citrus plants originated in south-east Asia and spread gradually to other parts of the world. The two most important orange growing / processing regions are Brazil and Florida in the US. The genus citrus belonging to the family Rutaceae comprises about 40 species which are distributed in India, China, Malaysia and Australia. Natural foods, especially citrus products, play a major role in human nutrition as excellent sources of antioxidants, including ascorbic acid, carotenoids, flavonoids and phenolics compounds. Citrus fruits contain biologically active compounds (or substances) which possess antioxidant activity. They are essential components of functional food as they help to prevent unwanted damage to cell membranes and other structures of the body by neutralizing free radicals. Antioxidants in citrus fruits possess antitumor activity. The peel which represents almost one half of the fruit mass contains the highest concentrations of flavonoids in the Citrus fruit. **Okwu et al., (2006)** reported that ascorbic acid – vitamin C – is the most important nutrient in citrus fruits, is essential for the synthesis of collagen, the most abundant protein in mammals. Collagen is the major fibrous element of skin, bone, blood vessels and teeth. A lack of vitamin C leads to scurvy which causes the loss of teeth, skin bleeding and ulcers. Vitamin C is sometimes suggested to have an anticancer effect because of its inactivation of free radicals in the body. Vitamin C is the most important antioxidant in citrus fruit, which protect the organism from oxidative stress. **Halliwell (2007)** stated that exposure of biological systems to xenobiotics pollutants, ionizing radiation or U.V. light and development of certain pathological conditions lead to oxidative stress, consequently increase production of oxy radicals. All are capable of reacting with membrane lipids, nucleic acids, proteins, enzymes and other

small molecules, resulting in cellular damage which is caused by free radicals appears to be a major contributor in aging and degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, compromised immune system, rheumatoid arthritis and brain dysfunction. Antioxidant action includes free radical scavenging, inhibition of lipid peroxidation, metal ion chelating ability and reducing capacity. The objective of the study was to assay and compare the levels of antioxidant enzymes in peels of ***C.sinensis***, ***C.reticulata*** and ***C. aurentifolia***.

Materials And Methods

Chemicals and reagents

All chemicals and reagents were analytical grade and general purpose reagents unless otherwise stated.

Procurement of samples

The following fresh citrus fruits a) *C.sinensis* (sweet lime) b) *C.reticulata* (orange) and c) *C.aurentifolia* (common lime) were purchased from local market of Allahabad, U.P., India.

Preparation of enzyme extracts

The fruit peels were washed thoroughly with tap water and rinsed with distilled water. A 10% homogenate (w/v) was prepared in the pre-chilled extraction buffer prescribed for each enzyme (usually the buffer used for assay of the enzyme) using mortar and pestle and filtered. The residue was discarded and supernatant used immediately for enzyme assay. The entire process was carried out at a temperature close to 4℃.

Ascorbic acid oxidase (EC.1.10.3.3)

The ascorbic acid oxidase activity measured was based disappearance of substrate (L-ascorbic acid) according to **Oberbacher and Vines** (1963) method. The reaction mixture contained 3 ml of substrate solution(ascorbic acid in phosphate buffer of pH 5.6) and 0.1 ml of enzyme extract (homogenised in phosphate buffer of pH 6.5). The change in absorbance was measured at 265 nm in 1min interval for 5min calculn

Catalase (EC.1.11.1.6)

Catalase activity was monitored by following the disappearance of H2O2, its substrate from the assay mixture, using the method of **Manonmani et al. (2009).** The standard reaction mixture (3.0 ml) contained phosphate buffer (46.7mM, pH 7.0) and H2O2 (15mM). The enzyme extract (100µl) was added to initiate the reaction. A control was prepared by adding buffer instead of enzyme extract. Tubes were shaken and the decrease in H2O2 concentration was measured as the decline in A240 during the first 10min after initiation of the reaction. Enzyme activity was expressed as changes in absorbance (Units ml-1).

Guaiacol Peroxidase (EC.1.11.1.7)

The activity of peroxidase was assayed by measuring the oxidation of guaiacol to form tetraguaiacol in the presence of H_2O_2 according to the method given by **Chanda and Singh (1997).** The reaction mixture contained 1ml each of the 8mM potassium phosphate buffer (pH 6.5), 1mM H_2O_2, 4mM guaiacol and enzyme extract. The change in absorbance at 470 nm due to the oxidation of guaiacol to form tetraguaiacol in the presence of H_2O_2 was measured.

Glutathione reductase (EC.1.6.4.2)

Glutathione reductase activity was done according to the method given by **smith et al., (1988)**. This assay was based on the increase in absorbance at 412 nm when 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) is reduced by GSH. The reaction mixture contained 1.0 ml 0.2 M potassium phosphate (pH 7.5) containing 1 mM EDTA, 0.5 ml 3 mM DTNB in 0.01 M phosphate buffer, 0.25 ml H_2O, 0.1 ml 2 mM NADPH, 0.05 ml glutathione reductase (1 U/ml), and 0.1 ml 20 mM GSSG. Glutathione reductase (extract) was kept in an ice-bath during preparation of the mixture, but its temperature equilibrated rapidly when it was added to the other components kept at room temperature. The reaction initiated by the addition of GSSG. The temperature was maintained at 24°C. The increase in absorbance at 412 nm was monitored using a spectrophotometer.

Polyphenol oxidase (EC.1.14.18.1)

The polyphenol oxidase (PPO) activity was measured by the increase in absorbance at 420nm with the oxidation of catechol as substrate according to the method given by **Liu et al., (2005).** The reaction mixture contained 1.0 ml of 0.1M catechol, 1.9 ml 0.1M phosphate buffer (pH 7.0), 0.1 ml of enzyme extract and incubated for 10 min at 30 ^{0}C . The increase in absorbance was measured at 420 nm with a spectrophotometer. One unit of PPO activity was expressed as $0.001\Delta A_{420}$ min^{-1} g^{-1} fresh weight.

Superoxide dismutase (EC.1.15.1.1)

The activity of superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) according to the method given by **Calatayud et al., (2002).** The reaction mixture contained 1ml each of 50mM potassium phosphate buffer (pH 7.8), 10mM methionine, 57µM nitroblue tetrazolium (NBT), 1.0µM riboflavin, 0.025 % (v/v) Tween-20 and enzyme extract. Then was thoroughly mixed and kept under fluorescent light of 30W for 15min placed 20cm away. Absorbance was recorded at 560 nm after the illumination period. In this assay, 1 unit of SOD was defined as the amount of enzyme necessary to produce a 50 % inhibition of the NBT photo reduction.

RESULTS AND DISCUSSION

Activity of Ascorbic acid oxidase in citrus peels

Activity of ascorbic acid oxidase in *C.aurentifolia* peels was to be maximum (201.0U/mg protein) whereas *C.sinensis* peels exhibited minimum activity (81.6 U/mg protein) according to **Table 1.**Since, literature on ascorbic acid oxidase activity in citrus fruits/peels is not readily available therefore; similar literature is quoted on various vegetables. **Shimada et al., (2008)** reported activity of ascorbic acid oxidase (units/100g) in broccoli (11.88), pumpkin (8.45), spinach (7.30), tingentsai (5.12), cabbage (4.96), carrot (4.22), taatsai (3.42), stalk garlic (3.27) and raddish (0.14).

Activity of catalase in citrus peels

Peels of *C.sinensis* had maximum

specific activity (1310.0 units/mg protein) whereas *C.reticulata* peels had minimum activity (820.4 units/mg protein) according to **Table 2.** Since, literature on catalase activity in citrus peels is not readily available therefore; similar literature on other plant samples is being quoted. **Yoruk et al., (2005)** reported a specific activity of catalase (29.43 U/mg proteins) in Van Apple fruit**. Manonmani et al**., **(2009)** reported 0.418 Units/min/g fresh tissue of catalase activity in healthy leaves of lime.

Activity of guaiacol peroxidase in citrus peels

The data presented in **Table 3** shows that *C.aurentifolia* peels exhibited maximum specific activity (4.82 units/mg protein) and *C.reticulata* had the least activity (2.98 units/mg protein). **Zia et al., (2011**) reported similar literature on guaiacol peroxidase in seeds of orange with 0.59U/mg activity.

Activity of glutathione reductase in citrus peels

The data presented in **Table 4** shows that *C.aurentifolia* peels exhibited maximum specific activity (0.369 units/mg protein) and *C.sinensis* had the least activity (0.031 units/mg protein). **Smith et al., (1988**) reported similar literature on crude extracts of soybean. From the above result it is concluded that *C.aurentifolia* peels possess high enzymatic antioxidants activity with the exception of catalase activity which is highest in *C.sinensis* peels.**Iannelli et al., (2002)** reported glutathione reductase activity (nkat per mg protein) of *Phragmites australis* leaves, roots and stolons 1.5 ± 0.3, 6.8 ± 0.8 and 4.3 ± 0.8 respectively.

Activity of polyphenol oxidase in citrus peels

Peels of *C.aurentifolia* exhibited maximum specific activity (9538.4 units/mg protein) and *C.reticulata* had the least activity (4154.9 units/mg protein) according to **Table 5**.**Wang et al**., (**2006**) reported similar literature on mango pulp (100 ± 8.8 % activity) with catechol as a substrate.

Activity of superoxide dismutase in citrus peels

The data presented in **Table 6** shows that *C.aurentifolia* peels exhibited maximum specific activity (28.56 units/mg protein) and *C.sinensis* had the least activity (7.60 units/mg protein). **Iannelli et al., (2002)** reported superoxide dismutase activity (U/mg protein) of *Phragmites australis* leaves, roots and stolons 12.5 ± 1.8, 35.9 ± 1.6 and 37.0 ± 1.0 respectively. **Rani et al., (2004)** observed 13.24 unit/mg protein of superoxide dismutase in orange fruit. The slight difference in results of *C. reticulata* (9.86 units/mg protein) maybe due to different assay method adapted and also due to peels which were used as a sample.

Table 1: Activity of ascorbic acid oxidase in citrus peels:

Citrus species	Enzymatic activity(mg of ascorbic acid oxidized/ml)	Specific activity (activity/mg protein)
C. reticulata	31.24 ± 0.02	110.0 ± 11.2
C.aurentifolia	26.13 ± 0.03	201.0 ± 18.05
C.sinensis	21.06 ± 0.12	81.6 ± 5.78

The values are MEAN ± S.D. (n = 3).

Table 2: Activity of catalase in citrus peels:

Citrus species	Enzymatic activity*		Specific activity (activity/mg protein)
C. reticulata	233± 0.78		820.4 ± 8.64
C.aurentifolia	128 ± 0.05		984.6 ± 7.01
C.sinensis	338 ± 1.20		1310.0 ± 9.56

The values are MEAN ± S.D. (n = 3).
*One unit of enzyme activity is defined as that amount of enzyme which breaks down 1µmole of H_2O_2 under the assay condition.

Table 3: Activity of guaiacol peroxidase in citrus peels:

Citrus species	Enzymatic activity*	Specific activity (activity/mg protein)
C. reticulata	0.8470 ± 2.1	2.98 ± 0.12
C.aurentifolia	0.6268 ± 1.71	4.82 ± 1.62
C.sinensis	0.8734 ± 2.31	3.38 ± 1.02

The values are MEAN ± S.D. (n = 3).
*U/mL
* The peroxidase activity was expressed as the rate of change of optical density (O.D.) per minute.

Table 4: Activity of glutathione reductase in citrus peels:

Citrus species	Enzymatic activity*	Specific activity (activity/mg protein)
C. reticulata	0.085 ± 0.31	0.299 ± 0.02
C.aurentifolia	0.048 ± 0.02	0.369 ± 0.03
C.sinensis	0.008 ± 0.42	0.031 ± 0.001

The values are MEAN ± S.D. (n = 3).
* U/mL
* One unit will cause the reduction of 1.0 mmole of DTNB to TNB at 25°C at pH 7.5.

Table 5: Activity of polyphenol oxidase in citrus peels:

Citrus species	Enzymatic activity*	Specific activity (activity/mg protein)
C. reticulata	1180 ± 2.51	4154.9 ± 3.76
C.aurentifolia	1240 ± 2.83	9538.4 ± 8.32
C.sinensis	1160 ± 1.98	4496.1 ± 3.54

The values are MEAN ± S.D. (n = 3).
* ΔA_{420}/min/g fresh wt.

Table 6: Activity of Superoxide dismutase in citrus peels:

Citrus species	Enzymatic activity*		Specific activity (activity/mg protein)
C. reticulata	2.802 ± 1.02		9.86 ± 2.08
C.aurentifolia	3.714 ± 2.04		28.56 ± 2.90
C.sinensis	1.962 ± 0.35		7.60 ± 1.02

The values are MEAN ± S.D. (n = 3).

***** One unit of 1 unit of SOD is defined as the amount of enzyme necessary to produce a 50 % inhibition of the NBT photoreduction.

It is widely accepted that consumption of fruits and vegetables is beneficial to health and contributes to decrease of the mortality rate of cardiovascular and other diseases. This study conducted for the assays of enzymatic antioxidants, ascorbic acid oxidase, catalase, guaiacol peroxidase, glutathione reductase and superoxide dismutase. The specific activity (units/mg protein) of enzymatic antioxidants *viz.* superoxide dismutase (28.56), guaiacol peroxidase (4.82), ascorbic acid oxidase (201), polyphenol oxidase (9538.4) and glutathione reductase (0.369) were highest in peels of *C.aurentifolia* with the exception of catalase which was maximum in peels of *C.sinensis*. The results obtained suggest metabolism of enzymatic antioxidants is more or less similar in citrus fruits peel whereas production of non-enzymatic antioxidants varies, indicates a potential for cheap and readily available natural source of antioxidants with health protective potential, which can be used in pharmaceutical, nutraceutical and food preparations. However, further studies required before these can be used as a source of antioxidants.

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IMPACT OF CLIMATE CHANGE ON GANGES RIVER WATER QUALITY AND ITS BIODIVERSITY IN HARIDWAR DISTRICT, UTTARAKHAND.

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Abstract

In today's world due to the excess growth in industrialization, urbanization, transportation is causing the degradation of the ecological balance through climate change. The major consequences of climate change are greenhouse effect, ozone depletion and epidemics which directly or indirectly affect the biological resources and life sustaining system of the nature. The aspect of climate change is felt all over the globe. The excess degradation of natural resources is causing climate change. The increasing concentration of greenhouse gases and deforestation has led to global warming which affects the weather, wind pattern and atmospheric conditions. Climate change affects water quality both through increased temperature and through changes in hydrological cycles. The increased water temperature affects ice coveras well as the rate of biogeochemical and ecological processes that determine water quality. The impact of climate change in ice cover period, thermal stratification and nutrient availability and longer growing seasons affects species composition and food web structures. Water temperature is one of the parameters that determine the overall health of aquatic ecosystems. River bank health is a term used to illustrate the ecological condition of a river bank or riparian zone. Health is more than just the plants and animals that live in a river bank, and the role of plants

in stabilizing the river banks and maintaining the river health. It depends on the diversity of habitats, plant and animal species, the effectiveness of linkages and the maintenance of ecological processes. Climate change affects the biodiversity through changes in water quality and quantity. Future climate change is expected to increase the rates of extinction, for species with restricted habitats or specialized niches. Changes in water levels and seasonal flows, also influences the feeding traits and spawning migrations and hence changes the breeding season for migratory birds. The biodiversity of River Ganga is unique as it synthesizes three very different eco-regions of India situated along climatic gradients, namely the Himalayas, the Gangetic plains and the Deltaic regions. The distribution of flora and fauna being largely dependent on the substrate, habitat and tropic status. This study is done to determine the impact of climate change parameters such as temperature and precipitation on the river water quality of Ganges River in Haridwar district, Uttarakhand which in turn affects the aquatic biodiversity of the river water. This study was done by collecting the past data of air temperature and precipitation and analyzing the impact of BOD and DO on biodiversity using the correlation coefficient. The study found the decrease and risk of extinction in aquatic biodiversity due to variation in the climatic (temperature and precipitation) and water quality parameters (BOD and DO).

Keywords: Climate change, water quality, biodiversity, Ganges river water, temperature, precipitation, BOD, DO

1.Introduction

The biodiversity of River Ganga is unique as it synthesizes three very different eco-regions of India situated along climatic gradients, namely the Himalayas, the Gangetic plains and the Deltaic regions. The distribution of flora and fauna being largely dependent on the substrate, habitat and trophic status.The biodiversity in the Ganga river may be grouped under seven heads, viz.: (i) Phytoplanktons (tiny free-floating living organisms that drift with the water and constitute the main autotrophic base of the food chain in the Ganga ecosystem); (ii) Periphytons (which, together with phytoplanktons, comprise 1176 Taxa of attached and free-floating algal forms); (iii) Zooplanktons (comprising 294 Taxa of largely macroscopic or assemblage of microscopic free-floating animals); (iv)Zoobenthos (comprising 73 families of insects including higher forms that group under rocks and boulders spending part of their life as larvae and those

which live and grow on soft substrate); (v) Fish (of 284 species plus 13 Chondrichthye species); (vi) Higher aquatic vertebrates (comprising Reptiles, Amphibians and Mammals that include 13 species of hard and soft turtles, besides the Gangetic dolphin, gharial, crocodile and porpoise); and (vii)Macrophytes (which are higher forms of plants that grow free floating or submerged in water bodies). Among these, periphytons, phytoplanktons and macrophytes are producers while zooplanktons, zoobenthos, fish and higher aquatic vertebrates are consumers of the food produced. Together, these micro- and macro-organisms, through their interplay with the abiotic environment, represent the ecological status of National River Ganga.

Rivers in India are losing their life. Many of them are running dry due to over abstractions while others are getting reduced to wastewater drains because of pollution.River Ganga is a lifeline to about 500 million people inhabiting its basin. The river Ganga provides water for drinking, domestic needs, agriculture, livelihoods, industrial use, fishing, boating, recreation, religious, cultural activities and for energy. Besides the humans, the river supports rich fauna and flora, including the endangered Ganges river dolphin, at least nine other species of aquatic mammals, reptiles (including three species of crocodiles along with one species of monitor lizard (Varanusbengalensis)) and different freshwater turtles. This mighty river also has the richest freshwater fish fauna anywhere in India.The ecosystem integrity and the goods and services offered by the river are getting adversely affected by the changes in its water quality and flow regime. Reasons are many: In the Himalayan reaches, until Rishikesh the flow in the river is threatened by water abstractions for existing and proposed hydropower projects. With many dams and barrages the river has become lean over the years, with 60% of its water being diverted before it enters the plains. While the impact on society and livelihoods is becoming more and more evident, itis also important to realise that the river habitats are controlled by physical processes—flow, water quality, sediment transport (Kaushal*et al*.2012).Managing (or mis-managing) the flow, can lead to significant changes to the biodiversity of rivers.Krishnamurthi*et al.* (1991) have identified 475 species downstream Rishikesh including 49types of tress with 16 having medicinal value with herbs 67%, shrubs 12%, climbers 6%,trees 10%, sedges 2% and native grass 3%. The riparian flora in Gangotri to Narora constitutes main vegetation as *Primula*, *Stellaria*, *Elatostema*, *Geranium*, *Rhododendron*, *Juniperus*and *Salix*. *Cedrusdeodara*borders the bed on both sides on the flood plain deposit. The important families are Poaceae, Asteraceae, Euphorbiaceae, Moraceae and Lamiaceae. The stretch Mirzapur

to Farakka has 40 macrophytes. The genera represented include *Eclipta*, *Polygonum*, *Ipomea*, *Rumex*, *Saccharum*, *Scirpus*and *Tamarind*.The total angiosperms in the middle and lower Ganga include 154 families and 680 genera (235 taxa of trees, 280 taxa of shrubs, 660 taxa of herbs, 680 taxa of weeds, terrestrial 832 and cultivated 289 taxa). The canopy trees include Saal (*Shorearobusta*),Teak (Tectonagrandis), Sheesham (*Dalbergiasissoo*), Mango (*Mangiferaindica*), Neem (*Azadirachtaindica*), Banyan (*Ficusbenghalensis*), Peepal (*Ficusreligiosa*), Jamun (*Syzygiumcumini*), Mahua (*Madhucalongifolia*) and Simal (*Bombax*sp.)

Various studies has been proposed about the impact of climate change on biodiversity and one of the major application of such studies has been proposed in the environmental planning.Climate change affects the biodiversity through changes in water quality and quantity.Future climate change is expected to increase the rates of extinction. (Thomas et al.2004), for species with restricted habitats or specialized niches.Changes in water levels and seasonal flows also influences the feeding traits and spawningmigrations (Poff et al. 1997) and changed the breeding season for migratory birds (Butler and Vennesland 2000).

India is a diverse country consisting of so many water bodies which makes it really essential to study one of the most important basin in India i.e. the Ganges and to understand the overall impact of climate change of the aquatic biodiversity.This study is done on Haridwar district, Uttarakhand which is the part of the Ganga basin to determine the impact of the climate change on the aquatic biodiversity. The study tries to determine the climate change impact on aquatic biodiversity in the area through the change in Ganga river water quality.

1.1 Site Selection

Ganga originates at 4,000 meters above the sea level in Uttarakhand from the southern slopes of the Himalayan region. It flows through almost five states of India and covers a distance of 2501 km before entering into the Bay of Bengal. In its long course it embraces many large rivers and tributaries of varied origin (Behera 1995,Rao 199).In Uttarakhand river after passing through Rishikesh enters Haridwar which is situated on the right bank of the river. The natural flow of the Ganges river has been obstructed due to building of barrages or dams either for irrigation or power projects. The Ganga basin is one of the most populous regions of the world due to this there is strong demand for natural resources such as water for domestic use and irrigation. There are 30 cities,70 towns and thousands of villages along the banks of the

Ganga.(WWF 2003).

The location of Haridwar on the Globe is on Latitude 29˚58'N and longitude 78˚10' E, while the height from sea level is 285.56 meters.The climate for this region is temperate, dry winter and warm to hot summer, with an ambient temperature for winter, summer and monsoon 9.3 to 16.3˚C, 26.8 to 40.4˚C and12.9 to 28.5˚C respectively.Annual precipitation here is over 31 centimeters.It is a city in Northern India on the bank of the Ganga River north east of Delhi. It is a Hindu pilgrimage centre. Hardwar lies along the Ganga River at the boundary between the Indo-gangetic plain (South) and the Himalayan foothills (North). The water supply of the Ganga system is partly dependent on the rains brought by the monsoon winds from July to October as well as on the flow from melting Himalayan glaciers in the hot season from April to June. The religious importance of Ganga may exceed than that of any other river in the world.

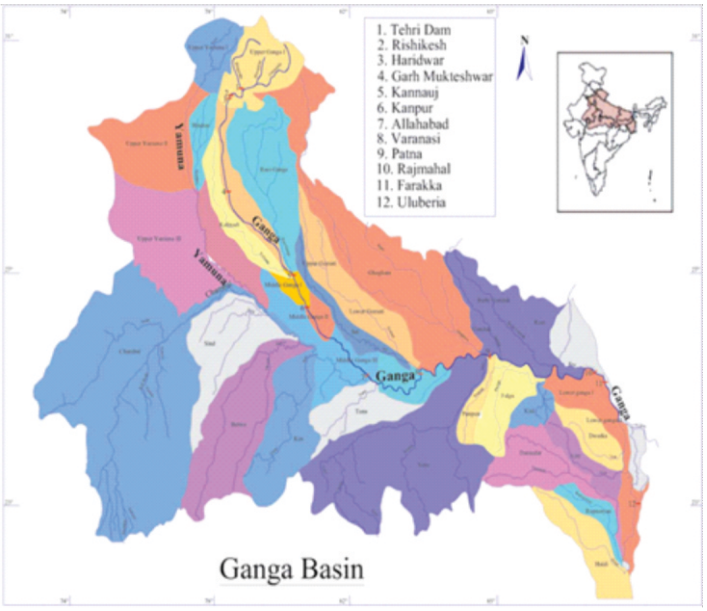

Figure 1 Ganga Basin

2.Materials and Methods

2.1Parameter Selection

The study collected monthly multi-parametric data of Haridwar district to understand impact of the climate change on aquatic biodiversity through change in water quality. The parameters used to study climate change are average monthly air temperature and total monthly precipitation (Moors et al., 2013). The parameters related to aquatic biodiversity used for measuring water quality parameters was biochemical oxygen demand and dissolved oxygen.(Missouri department of natural resources,1995).

2.2 Data collection and analysis

The method used to collect the data for climate change parameters was by performing the secondary literature review and data collected was from 2010 to 2011 for each month (India Water Portal, 2009). Statistical analysis likeKarl Pearson's correlationcoefficient (r value) was was carried out with the help ofMS Excel to find the relation betweenthe hydrological attributes and their impact on biologicalvariables.

3.Result and Discussion

The outline of the analysis of data which determines the climate change impact on aquatic biodiversity in the area through the change in Ganga river water quality are discussed here.

Table 1 Parameters and their Range & Average

Parameters	Range	Average
Temperature (°C)	16.0 – 27.5	19.35 ± 2.26
Dissolved oxygen (mg/l)	6.03 – 7.24	6.68 ± 0.34
Biochemical Oxygen Demand (mg/l)	83.0 – 167.0	148.69 ± 14.17

3.1 Concentration and Variation of climate change parameter

3.1.1 Temperature

The temperature is one of the most important ecological factor which controls the physiological behavior and distribution of the organisms. The catabolic energy released in the form of heat during the decomposition of organic matter and respiration also slightly added to the temperature. In the present study water temperature values ranged from 16.0 to 27.5 °C (Table 1) from the Figure2 shows that the monthly variation of temperature.The rates of biological and chemical processes depend on temperature. Aquatic organisms from microbes to fish are dependent on certain temperature ranges for

theiroptimal health. Optimal temperatures for fish depend on the species: some survive best in colder water, whereas others prefer warmer water. Benthic macro-invertebratesare also sensitive to temperature and will move in the stream to find their optimal temperature. If temperatures are outside this optimal range for a prolonged period of time,organisms are stressed and can die. Temperature is measured in degreesFahrenheit (F) or degrees Celsius (°C).For fish, there are two kinds of limiting temperatures the maximum temperature for short exposures and a weekly average temperature that varies according to the time ofyear and the life cycle stage of the fish species. Reproductive stages (spawning and embryo development) are the most sensitive stages.Temperature affects the oxygen content of the water(oxygen levels become lower as temperature increases);the rate of photosynthesis by aquatic plants; the metabolicrates of aquatic organisms; and the sensitivity of organismsto toxic wastes, parasites, and diseases.Causes of temperature change include weather, removal ofshading streambank vegetation.

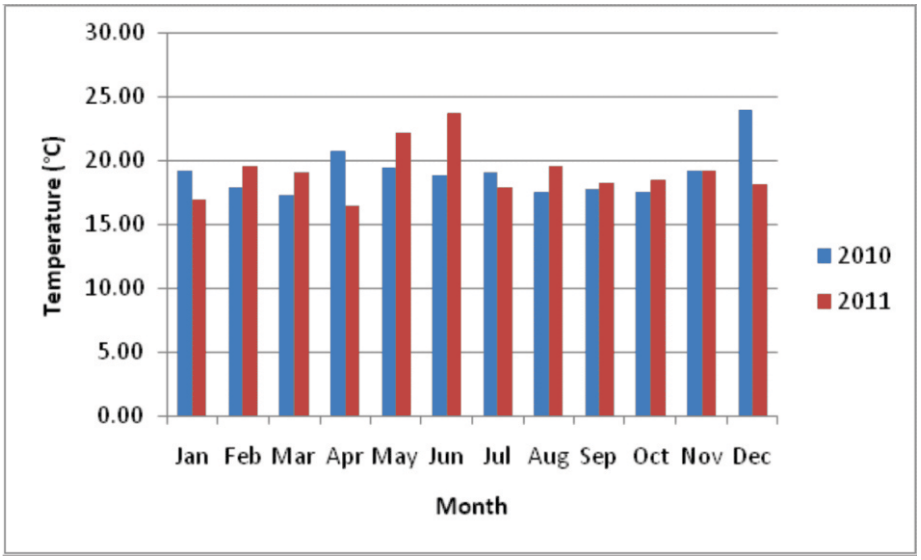

Figure 2 Monthly Variation of Temperature

3.1.2 Precipitation

It has been well established now that glaciers are melting at an accelerated rate in the Himalayan region. Secondly, the monsoon rainfall patterns are also changing. All these factors have major impact on the pattern of flows in the rivers and consequently on the aquatic biodiversity. It is measured in mm.In the present study precipitation values ranged from 1.0-400.0mm.

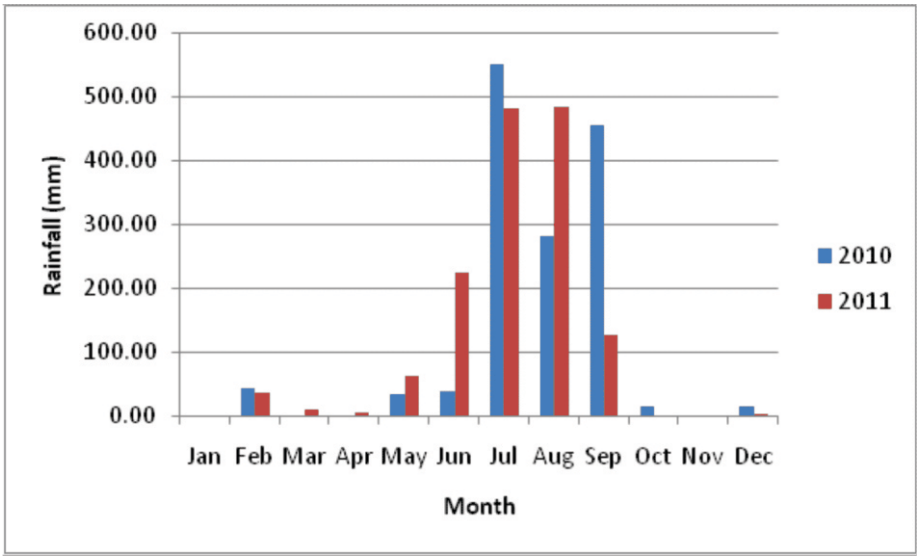

Figure 3 Monthly Variation of Rainfall

3.2 Concentration and Variation of aquatic biodiversity parameter

3.2.1 Dissolved Oxygen (DO)

The amount of Dissolved Oxygen, or DO, in water is expressed as a concentration. A concentration is theamount of in weight of a particular substance per a given volume of liquid. The DO concentration in astream is the mass of the oxygen gas present, in milligrams per liter of water. Milligrams per liter or mg/lcan also be expressed as parts per million, or ppm.The concentration of dissolved oxygen in a stream is affected by many factors:

Temperature: Oxygen is more easily dissolved in cold water.

Aquatic Plants: The presence of aquatic plants in a stream affects the dissolved oxygen concentration.Green plants release oxygen into the water during photosynthesis. Photosynthesis occurs during theday when the sun is

out and ceases at night. Thus in streams with significant populations of algaeand other aquatic plants, the dissolved oxygen concentration may fluctuated daily, reaching itshighest levels in the late afternoon. Because plants, like animals, also take in oxygen, dissolvedoxygen levels may drop significantly by early morning.

Usually streams with high dissolved oxygen concentrations (greater than 8 mg/l) areconsidered healthy streams. They are able to support a greater diversity of aquatic organisms. They aretypified by cold, clear water, with enough riffles to provide sufficient mixing of atmospheric oxygen into thewater.In streams that have been impacted by any of the above factors, summer is usually the most crucial time fordissolved oxygen levels because stream flows tend to lessen and water temperatures tend to increase.In general, DO levels less than 3 mg/l are stressful to most aquatic organisms. Most fish die at 12mg/l.However, fish can move away from low DO areas. Water with low DO from 2 – 0.5 mg/l are considered hypoxic; water with less than 0.5 mg/l are anoxic.Because the temperature of the stream can vary daily, and even hourly, it is important to factor out theeffect of temperature when analyzing the DO levels in a sample of water.DO values were found to be 6.68 ± 0.34 (6.03 - 7.24) mg/l as well as monthly variation of Dissolved Oxygen is shown in the Figure4.

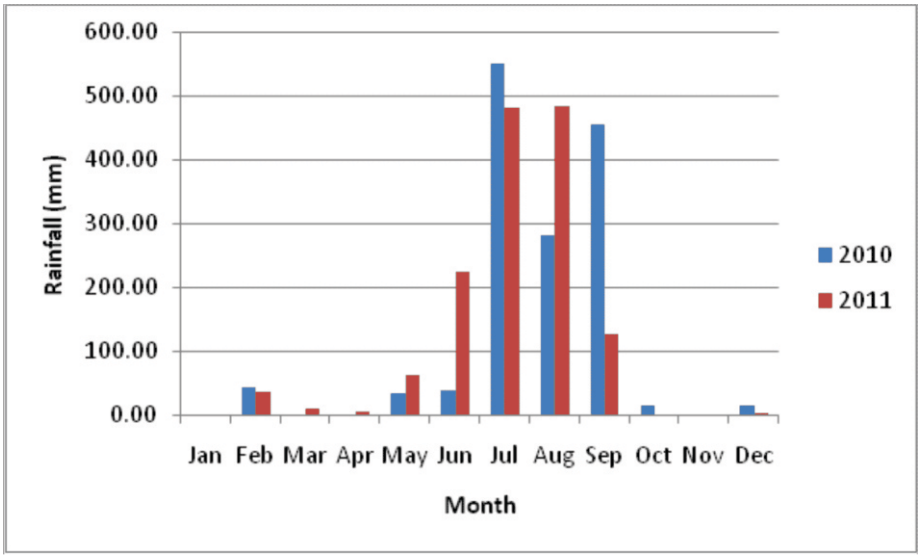

Figure 4 Monthly Variation of Dissolved Oxygen

3.2.2 Biochemical Oxygen Demand (BOD)

The Biological Oxygen Demand, or BOD, is the amount of oxygen consumed by bacteria in thedecomposition of organic material. It also includes the oxygen required for the oxidation of various chemicalin the water, such as sulfides, ferrous iron and ammonia. While a dissolved oxygen test tells you how muchoxygen is available, a BOD test tells you how much oxygen is being consumed.BOD is determined by measuring the dissolved oxygen level in a freshly collected sample and comparing itto the dissolved oxygen level in a sample that was collected at the same time but incubated under specificconditions for a certain number of days. The difference in the oxygen readings between the two samples inthe BOD is recorded in units of mg/l. Unpolluted natural waters should have a BOD of 5 mg/l or less. Raw sewage may have BOD levels rangingfrom 150 – 300 mg/l (1991, Stream keeper's Field Guide: Watershed Inventory and Stream MonitoringMethods) .BOD can be used as a gauge of the effectiveness of wastewater treatment plants. In the study period BOD were vary from 83.0 - 167.0mg/l which is within the permissible range (Figure 4) and statistical data analysis is given in the Table 1 (BIS, 1982 and WHO, 1993). All the water samples analyzed in the present study has BOD content within the prescribed limits.

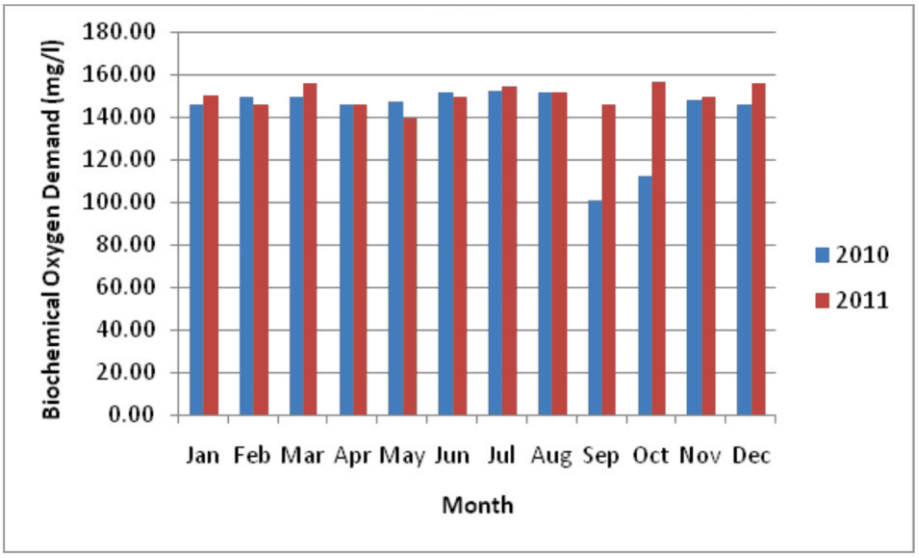

Figure 5 Monthly Variation of Biochemical Oxygen Demand

3.3 Correlation coefficient relationship among different Physico-Chemical parameters

In the present study, the correlation coefficient between each parameter pairs is computed to measure the strength & direction of the linear relationship. Correlation coefficient between any two parameters is calculated for parameter such as water Temperature, Precipitation, Dissolved Oxygen, Biochemical Oxygen Demand in Ganga River of Haridwar district. Considering the case of independent variable water Temperature, its impact on the dependent variables, like dissolved oxygen & biochemical oxygen demand is such that; the water temperature and the dissolved oxygen observed a weak uphill (positive) linear relationship of 0.28 in the year 2010, whereas in the year 2011 the same two parameters observed a very weak downhill (negative) linear relationship of -0.12. Similarly the water temperature and the biochemical oxygen demand observed a weak uphill (positive) linear relationship of 0.27 in the year 2010, whereas the same two parameters observed a weak downhill (negative) linear relationship of -0.34 in the year 2011. Now considering the case of independent variable Rainfall, its impact on the dependent variables, like dissolved oxygen & biochemical oxygen demand is such that; the rainfall and the dissolved oxygen observed a very weak downhill (negative) linear relationship of -0.11 in the year 2010, whereas in the year 2011 the same two parameters observed a close-to moderate downhill (negative) linear relationship of -0.42. Similarly the rainfall and the biochemical oxygen demand observed a weak downhill (negative) linear relationship of -0.26 in the year 2010, whereas the same two parameters observed a very weak uphill (positive) linear relationship of 0.14 in the year 2011. The discussion is shown below in Table 2.

Table 2 Correlation Coefficients Observed between Independent & Dependent Parameters

Independent Parameters	Dependent Parameters			
	Dissolved Oxygen		Biochemical Oxygen Demand	
	2010	*2011*	*2010*	*2011*
Water Temperature	0.28	-0.12	0.27	-0.34
Rainfall	-0.11	-0.42	-0.26	0.14

4. Conclusion

In the present study, all analyzed samples for physical and chemical properties of municipal wastewater discharge in Ganga River was found to be within desirable limits by various agencies.Thus, a predictive modeling

can be done in future to estimate the months in which aquatic biodiversity is at risk due to physico-chemical parameters. The study needs to be validated with the data collection from the concerned departments where the decrease in number of species, are reported.

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SIMULATION OF THE VARIOUS LAND USES AND LAND COVERS EFFECTS ON THE RATES OF RUNOFF AND INFILTRATION

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Abstract

Runoff measurement is the essential part of hydrology as it is necessary tool for the management of water and environmental management and can be defined as the draining or flowing off of precipitation from a catchment area to the surface channel. A catchment area may have variety of land uses and land covers as hillslope region, pasture area, irrigation area, exposed rock surfaces, forest area and residential/industrial area. Land use and land covers are known to effect the hydrological process like infiltration and runoff generation for the catchment area. Less infiltration is observed in urban area as compare to forest area and higher runoff is observed on bare earth as compare to forest area. Based on these land cover and land use conditions, the effect of altered soil characteristics on runoff is simulated using modified version of field process based infiltration techniques. Water does not only flow across the surface but filters down through the soil. A single infiltration model is not appropriate to mimic the mechanism of all type of exposed surfaces, it is expected for conceptually developed infiltration techniques for runoff estimation to enhance the performance of the rainfall-runoff model. The technique is based on the different layers and macropores-micropores interconnectivity as for different land uses and covers, it uses the different set of infiltration model and it estimates subsurface, interflow and return flow along with surface runoff. Present study reports the influence of some common type of land covers and land

uses on the runoff generation and focuses on different type of infiltration mechanism and its modification according to the Land use-Land covers.

Keywords: Land use, Land cover, Infiltration, Temperature, slope

1.Introduction

Infiltration is the movement of water into the soil from the ground surface by downward or gravitational flow through micro-macro pores and cracks. The rate at which it occurs known as infiltration rate (Osuji et al. 2010), which chiefly depends on soil type and the surface condition. A catchment basin is an extent or an area of land where surface water from rain, melting snow , or iceconverges to a single point at a lower elevation, usually the exit of the basin, where the waters join another water-body, such as a river, lake, reservoir, estuary, wetland, sea, or ocean. This Basin are so large that can be classified in different land uses and land covers significantly which generate different amount of runoff from the same precipitation.The infiltration rate and runoff process were found to be affected significantly by Land use and Land type (R. D. Sarkar et al. 2013). Land-use and Land type can be introduced by the macro-micro pore size and their interconnectivity of the existing soil matrix. It also changes the amount of abstraction and evaporation for different land. Experiments has shown the rate of infiltration will increase from pavement, bare earth, crop land, pasture and forest and similarly runoff will decrease in opposite sequence (R. D. Sarkar et al. 2013). Most of the theories either don't use the porosity as governing factor or use a constant porosity for different land type in a watershed that need to be develop based on field experiment and physical processes. Slope of the surface also influences the amount of infiltration and runoff rate (L. Jain et al. 2014). According to classical infiltration theories, either infiltration rate must be higher than precipitation rate (Horton, 1933) or it should be ponded before infiltration (Green Ampt, 1911). Experiment shows; even infiltration rate is higher, it starts runoff and runoff rate depends on slope surface and soil type (M. Weile et al. 2004).

In India, the maximum and the minimum temperature difference is approximate of 100℃ (-52 in LehLadakh to 50℃ in Alwar (Raj.) and in Delhi the Difference maximum and minimum temperature is approximate

50℃. (IMD, 2010). Hydraulic conductivity is the principle factor of infiltration rate which depends on the inter connectivity of pores. Experiment shows that higher temperature reduces the density of water and increases the hydraulic conductivity(G. J. Levy et al. 1989). For most of the Indian region this factor doesn't create the significant difference although in many regions, infiltration can be estimated more precisely.

Application of hydrology to the analysis of landform evolution and other scientific and land management problems requires realistic concepts of Infiltration process and runoff generation and their variation within drainage basins. These concepts need to be refined, developed and formalized through more vigorous combination of rigorously designed field experiments and realistic physically-based mathematical model.Present study deals with the different infiltration techniques for different land covers and land uses. It also represents the effect of temperature, slope and different soil characteristics on the infiltration process. Double ring infiltration is conducted for different land uses and then compared it with the existing infiltration techniques and developed infiltration mechanism and it is found the developed model is working efficiently but the application with the model is yet to be remained and part of further research work.

2. Methodology

The study consists of two parts, one is field experiment and the second is model development. Field experiment from Kulsi river basin (India) is executed by Chakma and Kumar (2002). Green Ampt method is modified by introducing the slope, temperature factor and porosity to make the model more generalize. Modified Green Ampt model is compared with the Horton model, Green Ampt model and field experimental observations of infiltration data.

2.1 Data Collection

The Experiment was conducted in theKulsi river basin. The Kulsi Basin has total drainage area 74030 Sq.KM, Basin length 86.46 KM and average rainfall inbasin is 1456 mm. Kulsi river basin chiefly consists of cultivated area, Dense/open forest, orchards,

pasture regions, mild slope region, hard surface and wasteland.Kulsi river basin consists of mainly clayey soil, sandy loam soil and clayey sandy loam soil.It can be observed the following characteristics of soil as shown in Table 1 (Chakma et al. 2002)

Table 1 Sandy Loam soil Characteristics

Saturated Hydraulic conductivity (cm/Hour) at 20°C	Soil Suction (cm)	Porosity (based on mean particle size)
2.99 -3.2	1.35-28	0.437

Temperature is measured by digital thermometer, and average temperature of the place is measured 16.5℃. Slope of the terrain is measured 7 ° to 12°by calibrated digital Angle-meter. Infiltration is measured by Double Ring Infiltrometer (outer ring 45 cm dia. and inner ring 30 cm dia.).Following are the key measurements of the experiments as shown in Table 2.

Table2 Significant measurement of Double Ring Infiltrometer experiment

Total time elapsed (min)	104
Initial infiltration rate (cm/min)	204
Final infiltration rate (cm/min)	26.4
Total (Cumulative) Infiltration (cm)	56.6

2.2 Model Development

Several models were developed to describe the infiltration of water from the soil surface in last recent years. The Green Amptequation is based on acceptable physical process and it'sseveral modified versions have been applied in many simulation models. It is based on the assumption that the ground surface is ponded by water and saturated hydraulic conductivity used in model but infiltration process starts as precipitation occurs on surface. Hydraulic conductivity should be function ofmoisture content or soil suction. In present model Hydraulic conductivity is estimated by Brook and Corey (1966) relationship between porosity, moisture content and saturated hydraulic conductivity as shown in equation 1.

$$K=Ksat\ (Wt-Wr)/(Wr))n$$

Where
K=Hydraulic conductivity, *Ksat*=Saturated hydraulic conductivity, *Wt*= moisture content at time t, η = porosity, *Wr*= Residual moisture content and *n*=Empirical

constant. Richard Equation is modified by Philip (1991) (Su Ninghu 2002) for the water movement and the infiltration into inclined surface. Philip concluded that the infiltration process can be independent of direction of slope, and dependent only on the normal direction of slope for the case of inclined surface. In this condition, Richard Equation can be modified into the form represented by Equation 2.

$$\frac{\delta\theta}{\delta t} = \frac{\delta\left[D(\theta)\frac{\delta\theta}{\delta z}\right]}{\delta z} - \frac{\delta K}{\delta\theta}\frac{\delta\theta}{\delta z}\cos\alpha \quad (2)$$

Where
D=Diffusion coefficient, θ= moisture content, z= depth of soil in normal direction of slope, t=time step, α= slope angle with horizontal, K=hydraulic conductivity

The Equation 2 suggests that the Cosine of slope angle can appreciably model the infiltration on inclined surface. It can be observed that inclined surface can provide more area for the same projection but it will reduce the total infiltration amount. In the study, slope is considered as constant, so it can be implemented directly with the conductivity in the model.

Temperature is the most effective meteorological factor to model a hydrologic phenomenon. The effect of temperature on water influences the hydraulic conductivity and the infiltration process as it changes the physical characteristics of fluid such as viscosity and density. Experiments have shown that increasing water temperature reduces viscosity which caused a significant increase in hydraulic conductivity (G. J. Levy et al. 1989). In general, models used the saturated hydraulic conductivity referenced to a standard temperature of 20℃ which can be used to estimate the corrected saturated hydraulic conductivity for a significant temperature (KsatT) by multiplying Viscosity correction factor. Viscosity correction factor is the ratio of viscosity of fluid at temperature 'T' (υ_T) to viscosity of fluid at temperature 20℃ (υ_20). Some values of correction factor are shown in Table 3 which are estimated by constant head permeability test for significant range of temperature.

$$Ksat20 = KsatT\upsilon\_\_T/\upsilon\_20 \quad \textbf{(3)}$$

Table 3 Viscosity correction factor

Temperature	4	8	12	16	20	24	28	30
Correction factor	1.5643	1.3819	1.2323	1.1057	1.0000	0.9083	0.8305	0.7956

Land use-land type can be considerably defined by soil texture, soil strata depth, average bulk and dry density; physically they all lead to the estimation of porosity of the soil matrix and the inter-connectivity between different sized pores. Effective porosity has been considered as prime factor to model the infiltration in such as including Green-Ampt model, although porosity will be different for same soil if the land use is different. Macropores and micropores value for the different landuse-land type is presented in the Table 4 (R. D. Sharma et al. 2013). The effectiveness of both the micro-macro pores can be estimated by calibrating the model. In the presented model; macro-micro pores are kept with equal weightage.

Porosity= (micropores+macropores/2

(4)

LULC type	Micropores	Macropores
Paddy field	0.371±0.01	0.030
Tea garden	0.355±0.005	0.035
Grassland	0.475±0.005	0.027
Jhum cultivation	0.375±0.005	0.055
Bamboo cultivation	0.425±0.005	0.040
Moderately dense forest	0.375±0.005	0.035
Sparse forest	0.375±0.005	0.053±0.0025
Mixed forest	0.375±0.005	0.052±0.0035

An analytical model is developed based on Green Ampt equation including all the mentioned factors to mimic the relatively more real system as shown in Equation 5.

$$F(t) = K(W,t).\frac{\upsilon_{20}}{\upsilon_T}.\cos\alpha + (Wt - Wt-1).\psi(W,t)\log\left[1 + \frac{F(t)}{\psi(Wac,t).(Wt - Wt-1)}\right]$$

Where
F(t) = Cumulative infiltration at time t (cm), K=hydraulic conductivity (cm/min), W_i=Moisture content at time t, ψ=Soil suction (cm), υ_20/υ_T = Viscosity correction factor, α=slope angle.
Soil suction is function of pore size, porosity and moisture content; and estimated by Nissar (1966)empirical relationship between moisture content and soil suction (L. Jain et al. 2014).

$$\psi=(a(\eta-w)^b)/w^c \quad \textbf{(6)}$$

Where
W=Moisture content, η=porosity and a, b, c= empirical constant based on soil pore size and texture

1. Results

The results of the model simulation run for the study area Kulsi river basin is compared with the experimental data and classical infiltration models (Horton equation, Green Ampt equation). A statistical analysis is also performed to evaluate all the models.

3.1 Comparison of cumulative infiltration

The developed model is used to compute the cumulative infiltration at every time step t for the single set of experimental data of Kulsi river basin. Evaporation loss is neglected during the experiment and mean temperature is observed as 16.5^{0}C at experimental field. Constant infiltration rate was observed till time step of 104 minutes during experiment; therefore the model is run for 104 minutes and compared with the Horton model and Green Ampt equation. The model is run with the average porosity of 0.437 and residual moisture content 0.036. (Figure 1).

Model analysis shows that all the models are performing better considerably well for the single set of infiltration data because the temperature difference is only 4C ^{0} from the temperature used in the classical models but for the large watershed area, the difference would be more significant. According .to Nash Sutcliffe, PBIAS and coefficient of correlation, the developed model is performed slightly better and R^2 value of Horton equation is found insignificantly better as shown in Table 5.

Table 5 Statistical comparison of models

Model	Nash Sutcliffe	PBIAS	Coefficient of correlation	R^2
Horton	0.92	0.12	0.995	0.997
Green Ampt	0.80	0.05	0.983	0.986
MGA	0.93	0.04	0.997	0.985

3.2 Comparison of infiltration rate

Infiltration rate for experimental data at each time step is analyzed and compared with Green Ampt model, Horton model and developed model. Statistical analysis indicates that the developed model has performed better than the Green Ampt equation but the Horton model has shown better statistical similarities as shown in Figure 2.

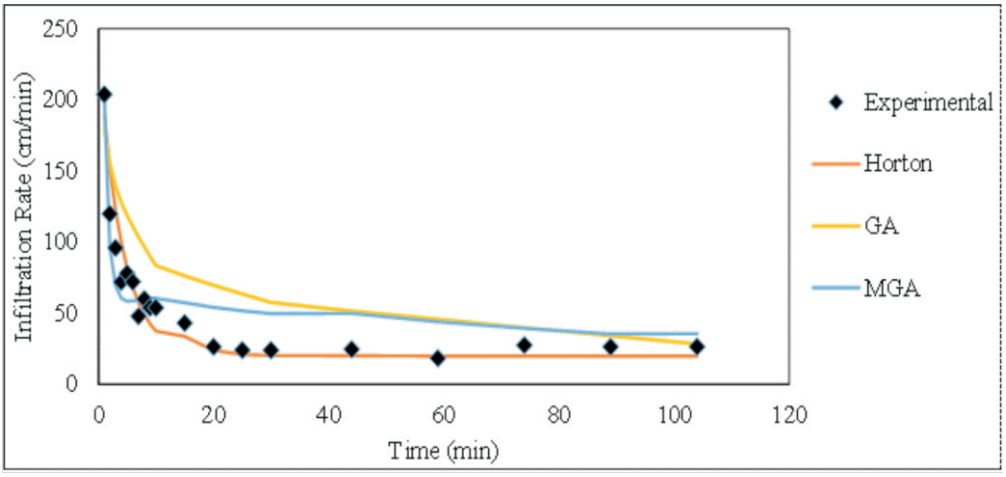

Figure 2 Comparison of infiltration rate

Initially from 0 minutes to 20 minutes variation in soil suction and hydraulic conductivity in Green Ampt model, prepared it for more realistic scenario. Soil suction gradually decreases when the soil matrix reaches to saturation level as shown in Figure 3. Statistical comparison of infiltration rate is presented in Table 6.

Table 6 Statistical comparison of infiltration rate

Model	Nash Sutcliffe	PBIAS	Coefficient of correlation	R^2
Horton	0.9	0.091	0.96	0.92
Green Ampt	0.34	0.521	0.9	0.82
MGA	0.84	0.096	0.93	0.87

3.3 Variation of model parameters

Developed model has shown the variation of moisture content, Soil suction and Hydraulic conductivity at each time step to understand the infiltration process significantly. As infiltration starts; moisture content increases in soil matrix from initial moisture content to degree of saturation (Figure 3). Soil suction decreases from 18 cm to 0.02 cm with the increment of soil moisture content to saturation as shown in Figure 4.

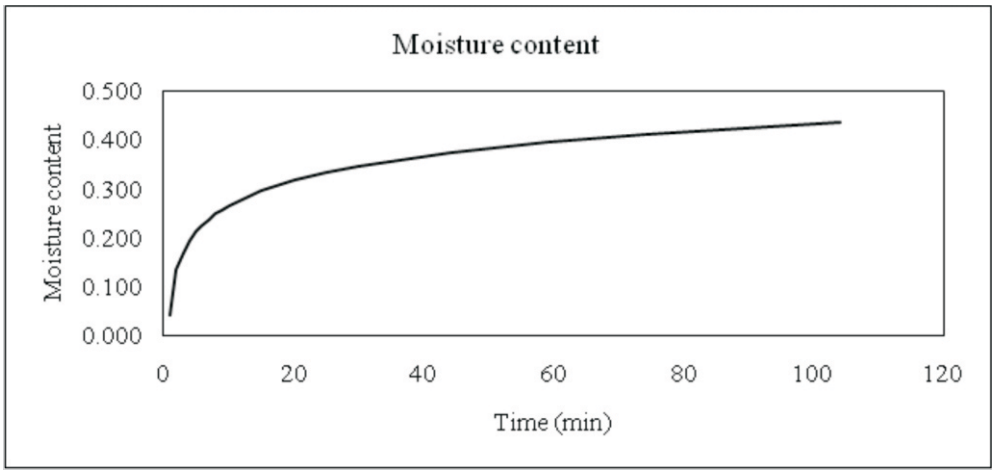

Figure 3 Variation in moisture content

Saturated moisture content is nearly equal to average porosity of the soil matrix, which is 0.437. Soil suction for sandy clayey soil experimentally measured 1.35 to 28 (Todd and Mays 2005) which validates the result.

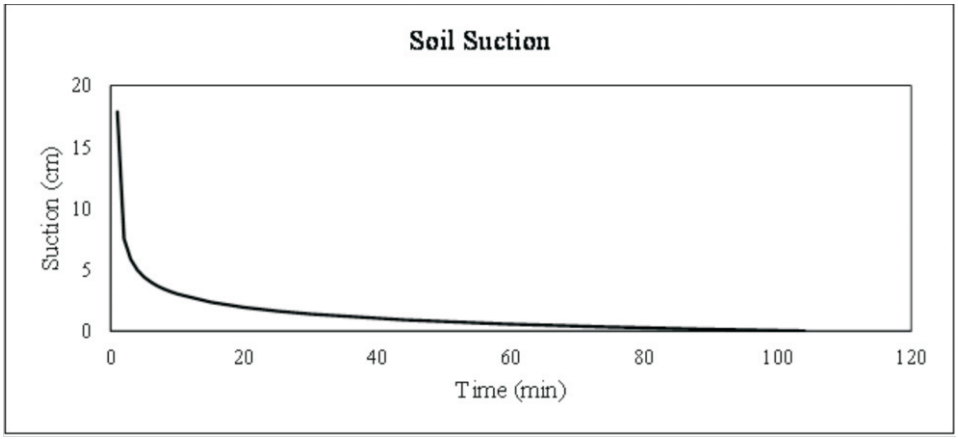

Figure 4 Variation in Soil suction

Hydraulic conductivity increases as moisture content increases and soil suction decreases with each time step and varies the infiltration process simultaneously. When moisture content reaches to saturation level, hydraulic conductivity reaches its maximum value called saturated hydraulic conductivity (Figure 5).

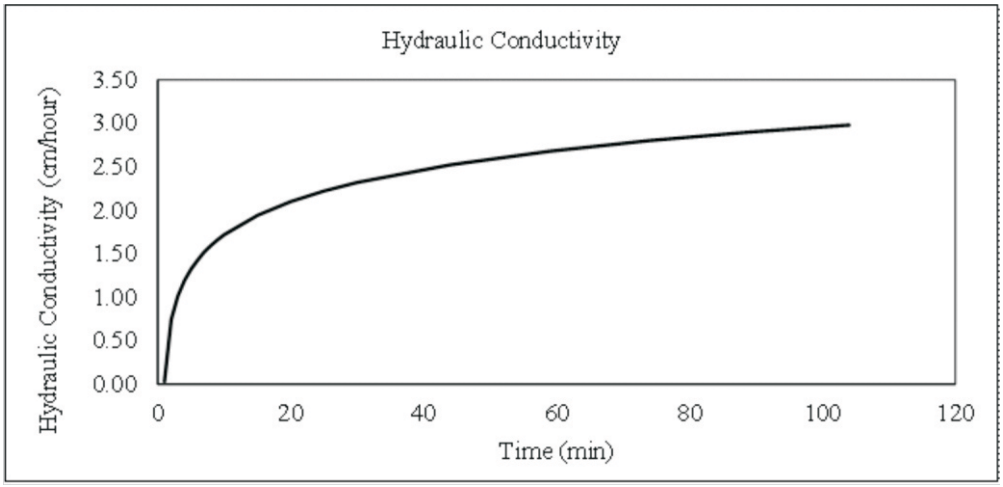

Figure 5 Variation of Hydraulic conductivity

1. Conclusion

The results of the developed model have been analyzed and compared with the Horton model, Green Ampt model and observed infiltration data. It is concluded that developed model performed reasonably well. Comparison suggests the suitability of infiltration model in estimating the cumulative infiltration involving temperature, soil-type, land use and slope of concerned region. The following conclusion can be drawn from the study-

1. As temperature increases, it decreases the viscosity of water and increases the hydraulic conductivity which leads to considerably additional water infiltration for the larger area.
2. Horton has performed better estimation of infiltration rate because conceptually it depends on single parametric constant and can easily analyze single data set.
3. Maximum macropore and minimum macropore size define the volume of infiltered water and rate of infiltration.

The study has identified that the variation in meteorological factors, landuse-landtype and slope affects the infiltration process significantly. The developed model can be used for homogeneous soil type including landtype factor and constant average slope. Experimental depth of soil matrix is measured 10-15 cm which is considerably small and need to modify

infiltration rate at various depth. The model has still scope of improvement as it needs to be analyze for more field condition and an Abstraction factor with Evaporation loss is needed to enhance the efficiency of the model.

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LARGE SCALE LABORATORY INVESTIGATION AND SIMULATION OF FATE AND TRANSPORT OF LNAPL PLUME IN VARIABLY SATURATED SUBSURFACE

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Abstract

The aim of this study to investigate the fate and transport of a dissolved phase light non aqueous phase liquids (LNAPLs) plume in variably saturated zone using large scale laboratory and simulation experiments. The experiments were conducted in a two dimensional tank setup constructed of one piece of thick stainless steel. The front cover is made of a thick glass sheet and the space between the two walls is packed with a sandy soil of particle size 0.5-1 mm free from organic matter. The solute flux of 100 ml/h was provided as the point source of 50 ppm concentration of dissolved toluene, an LNAPL representative, from one side of the tank. The water flux was taken out from the tank at the other end to maintain the constant water table. The samples of soil water were collected using ports having equal horizontal spacing of 13 cm from two horizontal layers situated 60 cm apart vertically in the steel box. The collected samples were analyzed in triplicates by GC- MS. The laboratory investigation were compared with the simulation experiments using HYDRUS 2D by incorporating biodegradation as a sink term. The spatial movement of LNAPL showed different decreasing trends in lateral and vertical directions at observation points away from the source location. The breakthrough curves showed the fast degradation rate at initial time which started decreasing with progression in time before reaching to the equilibrium. The equilibrium peak concentration of the LNAPL decreases while going towards the outlet showing biodegradation of the considered hydrocarbon in the soil-water system. The results of this study may assist in applying bioremediation in field and for decision making related to planning of industrial locations.

Keywords: Groundwater contaminant modeling, LNAPL, 2D Laboratory Investigation, Biodegradation, HYDRUS 2D,

1. Introduction

Soil-water resources are the fundamental supportive natural resources to life on the earth. Whereas the increasing pollution through different sources likes industrial effluent, municipal waste, etc. becomes threats to the soil-water resources. Petrochemical are the most common pollutants to degrade the soil-water systems (Seeger et al. 2011) and found to be toxic and carcinogenic at high concentrations (Nadim et al. 2000, Zhang et al. 2010). The accidental spills and leakage from the industrial units, and underground storage are the main sources of such pollutant to (sub)-surface (Bento et al. 2005). Petrochemicals are popularly referred as non-aqueous phase liquids (NAPLs) due to their immiscibility with water. On the basis of density, these pollutants are classified in light non-aqueous phase liquids (LNAPLs) and dense non-aqueous phase liquids (DNAPLs) (Yadav et al. 2013). The LNAPLs movement in the subsurface is mainly dominated by advection and hydrodynamic dispersion mechanisms (Yadav et al. 2011). Biodegradation of these pollutants also take place during their movement in subsurface. In subsurface, the environmental variables like temperature, soil moisture content and fluctuation of water table over times affects the fate and transport of these pollutants (Dobson et al. 2007).

Many investigations were conducted in small scale batches but such experiments only useful to observe the biodegradation rates, adsorption coefficient, and Henry's coefficient of the pollutant in the laboratory. In the batch systems the soil-to-solution ratio is low and there is no flow component applied so it cannot accurately represent the real field situation of the aquifer. Mesocosm experimental studies using columns have been attempted to realize the dynamic flow component under controlled laboratory conditions. These experiments provide a link between microcosms and field scale to observe movement of the pollutant in small scale (Sturman et al. 1995, Yadav et al.2012). Whereas most of these experiments focuses on the single parameters characterization. Therefore, the large scale experiments are needed for incorporation of different governing parameters to domain.

Very few researches were investigated the large scale fate and transports using experimental information based simulation under variable environmental condition. Therefore, in this study we investigated the fate and transport of a dissolved phase LNAPL plume in variably saturated zone using large scale laboratory and simulation experiments.

2. Methodology

2.1 Experimental Media

The fine sand having particle size 0.5-01 mm used as experimental media. The physiochemical properties of the experimental media describes in the table 2. The India standard (IS) sand 650 grade-II having less than 1 mm & greater than 0.5mm particle distribution sizes were used. Before use in tank, sand were washed and oven at 100^0C for 24 hours. The oven dried sands was used in the final setup of the sand tank.

Table 2. Physical and chemical properties of the experimental porous media.

Porous media type	Sand (g hg^{-1})	Silt (g hg^{-1})	pH	EC	OM[a]	Bulk Density (g/cm^3)	Dimension (l*h*w) cm
Sand	92.8	4.2	5.5	4.9	0%	1.65	150×60×10

2.2 The LNAPL Representative

The analytical standards for toluene were purchased from Sigma New Delhi INDIA. The physiochemical properties and the chemical structure of these chemical are shown in Table 1. The 50 ppm dissolved phases were prepared by adding 10 ml of the pure phase LNAPLs in 990 ml of miliQ water and shaked 24 hours using magnetic stirrer.

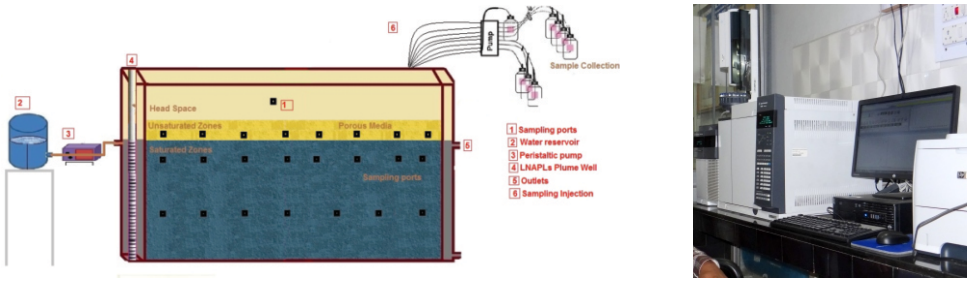
Table 1. Physiochemical properties and structures of Toluene

Parameters.	Toluene
1. Molecular formula	$C_6H_4(CH_3)_2$
2. Molecular weight	106.2
3. Density, [g cm^{-3} (at 20^0C)]	0.8611
4. Viscosity, [cP (at 20^0C)]	0.648
5. Water solubility [mg L^{-1} (at 25^0C)]	156
6. Diffusivity [cm^2s^{-1}]	$7.2*10^{-6}$
7. Structures	

2.3 Laboratory setup & Method

The experiments were conducted in two dimensional tank setup constructed of one piece of thick stainless steel formed into a box with inner thick sheet (figure 1b). The front cover is made of a thick sheet and the space between the two walls is packed with a sandy soil of particle size 0.5-1mm free from organic matter. A solute reservoir was installed at the same water table. The solutes were taken by the peristaltic pump (Model RH-

P100VS-100-2H) having the both side jointed viton tubing. The viton tubing is recommended for the LNAPLs studies. The water flux of 100 ml/h was provided as the point source of 50 ppm concentration of dissolved toluene, an LNAPL, to the sand from one side of tank. The same water flux was taken out from the tank at the other end to maintain the constant water table and to represent the actual groundwater flow scenario in unconfined aquifers. The samples of soil water were collected using syringe (0.55×25mm) from ports having equal horizontal spacing of 13 cm from two horizontal layers situated 60 cm apart vertically in the steel box. The samples were directly injected into 1.5 ml vials (Agilent vials) having air tight caps without any air contacts. The air phase samples were also collected from the head space ports situated in top portion of the steel box. The samples were immediately analyzed by the GC-MS (figure 1b). In this experiment, the entire 2D tank setup were air tight and there were no any single open space except the outlet at other side of the tank. Therefore, the volatilized concentrations were the actual in head space and there were no loss of concentration from the tank in any phase of LNAPLs.



a). b).

Figure 1: Laboratory setup, a). Schematic diagram of the 2D sand tank setup, b). GC-MSD analysis of collected samples.

2.4 Sample Analysis

For the analysis of the hydrocarbons especially LNAPLs, the Gas Chromatography recommended using the FID or MS detector. In this study, the collected water samples were analyzed by Gas Chromatography

Mass Spectrometer (GC-MS) model Agilent-7890B showed in figure 1b. The calibrations were taken first using the different know concentration and the toluene specific command were set in the GC. The samples were placed in automatic sampler having 16 vials ports. The 5 minutes were taken as analysis time and the analyzed peak were filtered. The peak area were gives the concentration of the samples. The triple analysis of each samples were considered and the mean values are using for the further simulation.

2.5 Simulation Experiment

The groundwater flow was simulated using the mixed form of Richards's equation as:

$$\frac{\partial\theta}{\partial t} - \nabla . D(\theta)\nabla\theta - \frac{\partial K}{\partial z} = 0 \qquad (1)$$

Where $\frac{\partial\theta}{\partial t}$ is the specific moisture capacity

function, K(h) is the unsaturated hydraulic conductivity, $D(\theta)=K(\theta)/C(\theta)$, is the unsaturated diffusivity, z denotes the vertical dimension. Furthermore, for the multi-dimensional advection-dispersion mechanisms of solute transports in subsurface with a decays kinetics was simulated using the classical advection dispersion equation as

$$D_x \frac{\partial^2 C}{\partial x^2} + D_y \frac{\partial^2 C}{\partial y^2} + D_z \frac{\partial^2 C}{\partial z^2} - V_x \frac{\partial C}{\partial x} - \frac{r}{n} = \frac{\partial C}{\partial t} \qquad (2)$$

Where $D_{x,y,z}$ = hydrodynamics dispersivity in x,y,z, direction, V_x is the uniform velocity and r is the biodegradation kinetic decay rate.

The simulation experiments were evaluated using the laboratory based information to the most cited subsurface model HYDRUS 2/3D. The HYDRUS 2/3D is graphical user interface tool coded on above mention governing equation of the variably saturated subsurface water flow and solute transport (Yu and Zheng 2010). The simulation projects were created in 2D general types of geometry having vertical plane XZ. The tank size domain was graphical edited as X: 10 cm, Y: 150 cm, Z: 90cm and simulated for water and standard solute transport. The van-Genuchten-Mualem single porosity model was selected for the domain. The van-Genuchten parameters were used as material properties of the water flow for single sand media showed in the table 3.

Table 3. The ven-Genuchten parameters for sand media.

Sr.No	Media	Qr [-]	Qs [-]	α [1/cm]	n [-]	Ks[cm/hr]	L[-]
1	Sand	0.045	0.43	0.145	2.68	29.7	0.5

Similarly, Space discretization followed the Galerkin finite elements approaches and time discretization followed Crank-Nicholson scheme for the solute transport. Upper boundary water flow conditions were atmospheric boundary and the lower boundary water flow were No flux boundary. Similarly, the solute transport upper boundary conditions were concentration flux BC and the lower were zero concentration gradient (figure 2). The observation points were graphically edited in two horizontal layers having same dimensional domain as in 2D tank. Finally the mass balances were calculated for the study domain.

3. Results and Discussion

The relative concentration of toluene was plotted as a function of time at different depths, known as breakthrough curves (BTCs), for 2D study domain. The observed BTCs for the study domain are shown in Figure 2 a, b using the dot points of different colors for different observation points. In figure 2a, the breakthrough curve shown for the 1 horizontal layers having nine ports with 13 cm distance to others. The BTCs also represented the time of arrival of LNAPL plume at different location of the tank with their relative concentration profile. It was observed from the breakthrough curves that the equilibrium concentration time for observation ports was higher for ports nearby sources as compared to the others which clearly indicated the amount of biodegradation happening in the soil mass. The difference in ports BTCs with respect to sources BTCs gives the degradation rate and showed the fast degradation rate at initial time and start decreasing with progression in time before reaching to the equilibrium. The equilibrium peak concentration of the LNAPL decreases while going towards the outlet showing spatial biodegradation in the define study domain. The shape of the BTCs for all ports was same at equilibrium peak concentration.

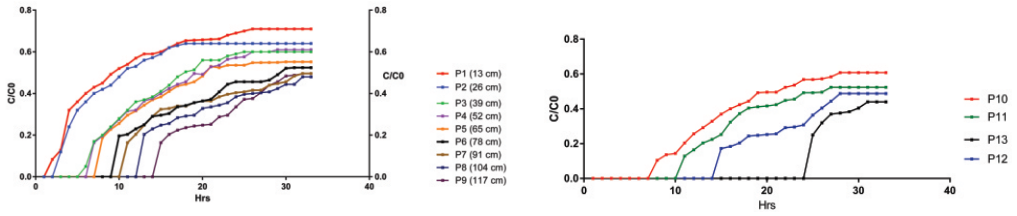


Figure2. Experimental breakthrough curve of observation ports a) P1-P9 (1st layer) b) P10-P13 (2nd layer)

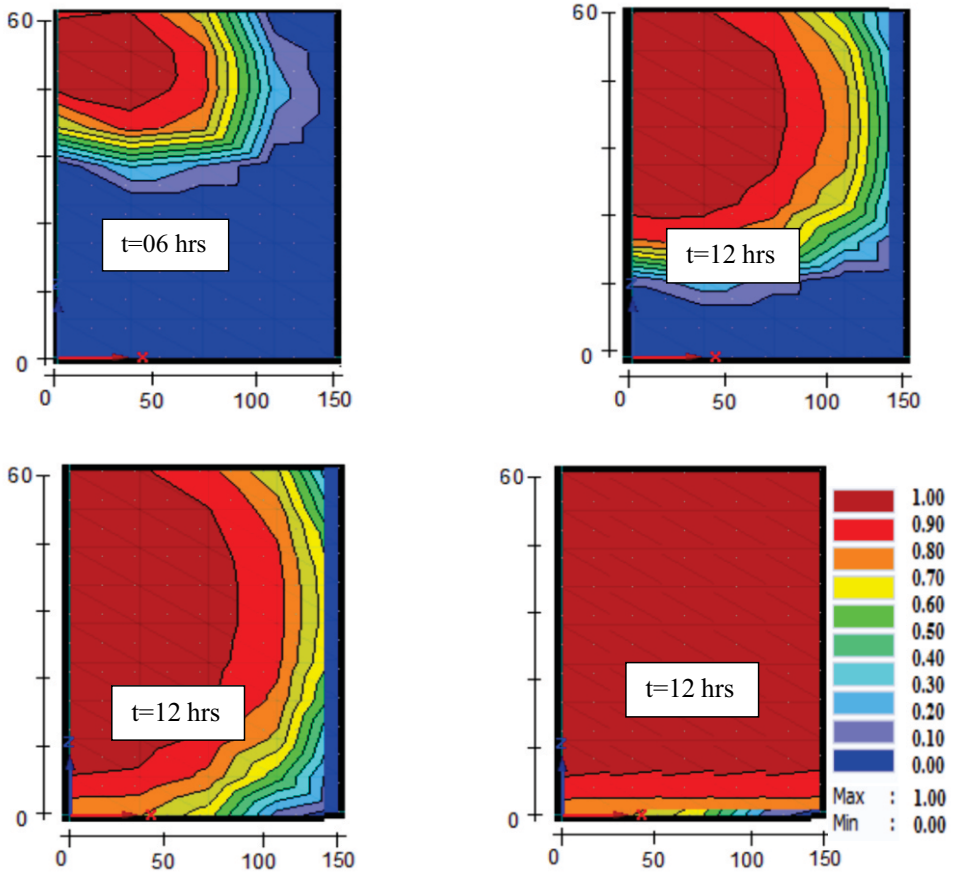


Figure3. Simulated concentration profile of the domain at time, t=06hrs, t=12 hrs, t=18hrs, t=24 hrs.

The laboratory investigated BTCs were compared with the simulation experiments using HYDRUS 2D by incorporating biodegradation as a sink term. The simulated spatial plume distribution in domain represented in figure 3 at time 06 hrs,12 hrs,18 hrs,24 hrs. The concentration profile curve of the domain showed the increasing concentration as time advance. After 24 hrs the entire domain showed at equilibrium concentration. Similarly in simulated BTCs also, spatial movement of LNAPL showed different concentration decreasing trends in lateral and vertical directions at observation points away from the source location. The simulated BTCs showed similar time of arrival of the plume at different observation points and similar concentration profile throughout the domain (figure3a,b).

3. Conclusions

The intent of this study was to investigate the fate and transport of dissolved LNAPL plume in variably saturated zone. The experiments were conducted using two dimensional sand tank and simulation in HYDRUS 2/3D. The simulation underpinning the laboratory experiment incorporated the biodegradation rate as sink also. Therefore, the entire experiment focuses on the spatial movement of the plume in the domain which represented the actual scenarios as in field. It was observed from the breakthrough curves that the equilibrium concentration time for observation ports was higher for ports nearby sources as compared to the others. Both, laboratory and simulated results showed the different concentration decreasing trends in lateral and vertical directions at observation points away from the source location. The breakthrough curves showed the fast degradation rate at initial time and start decreasing with progression in time finally reaching to the equilibrium. The equilibrium peak concentration of the LNAPL decreases while going towards the outlet showing biodegradation of the considered hydrocarbon in the soil-water system. This study on fate and transports of LNAPL may assist in ecofriendly and cost-effective remediation technologies for in situ treatments of polluted soil water resources.

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STATUS OF NON-ENZYMATIC ANTIOXIDANTS AND ANTIOXIDANT ACTIVITIES FROM CITRUS FRUITS PEEL.

Akhilesh Bind
Archana Singh

Abstract

Three species of citrus fruits were chosen to investigate and evaluate levels of non-enzymatic antioxidants and to assess free radical scavenging activity of above mentioned citrus species by DPPH scavenging method and NO scavenging activity in peels of C.reticulata, C.sinensis and C. aurentifolia. The results indicate that amongst these 3 citrus fruits peels; the levels non-enzymatic antioxidants varied amongst the species. Ascorbic acid and total phenols were highest in peels of C.sinensis, flavonoids content was highest in peels C. aurentifolia and carotenoids content was maximum in C. reticulata peels. Antioxidant activity of each extract increases with concentration. Ethanolic extracts of these citrus fruits peels exhibited maximum antioxidant activity except in peels of C.sinensis. Low IC_{50} values in free radical scavenging activity of the ethanolic extracts indicate a potential for cheap and readily available natural source of antioxidants with health protective potential, which can be used in pharmaceutical, nutraceutical and food preparations.

Keywords: Antioxidant activity, Non-enzymatic antioxidants, DPPH, NO, Citrus peels.

Introduction

Free radicals have been claimed to play an important role in affecting human health by causing several diseases including cancer, hypertension, heart attack and diabetes. These free radicals are generated during metabolism. Exogenous intake of antioxidants can help the body scavenge free radicals

effectively **(Halliwell, 1994).** Citrus fruits are the world's most popular fruits. The genus citrus belonging to the family Rutaceae, originated in South-east Asia and spread gradually to other parts of the world. Natural foods, especially citrus products, are an excellent sources of ascorbic acid, carotenoids, flavonoids and phenolics compounds which possess antioxidative, anti-inflammatory and anti-carcinogenic properties **(Zvaigzne *et al*., 2009).**Ascorbic acid – vitamin C – is the most important nutrient in citrus fruits, is essential for the synthesis of collagen, the most abundant protein in mammals. Collagen is the major fibrous element of skin, bone, blood vessels and teeth. A lack of vitamin C leads to scurvy which causes the loss of teeth, skin bleeding and ulcers. Vitamin C is sometimes suggested to have an anticancer effect because of its inactivation of free radicals in the body. Vitamin C is the most important antioxidant in citrus fruit, which protect the organism from oxidative stress (**Okwu *et al*., 2006).** The intensive colour of citrus fruits is mainly due to substances called carotenoids. The main carotenoids responsible for the orange colour of orange are α-carotene, zeta-antheraxanthin (yellowish), violaxanthin (yellowish), β-citraurin (reddish orange), and β- cryptoxanthin (orange) (**Wong *et al.,* 2006).** Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities **(Van Acker *et al.,* 1996).** Flavonoids are antioxidants that interfere with lipid oxidation, reduce cholesterol and protect blood vessels from thrombosis. Studies in Holland have shown that a special diet containing flavonoids improved decreased defenses against free radical cell damage and lipid oxidation, or the risk of myocardial diseases and infarction. There are clinical and epidemiological evidences to indicate that polyphenol reduce the risk of chronic diseases. This means that they are important substances for genetically based long-lasting life (**Tripoli *et al*., 2007).** The peel which represents almost one half

of the fruit mass, contains the highest concentrations of flavonoids in the citrus fruits **(Manthley and Grohmann, 2001 and Anagnostopoulou *et al.*, 2006).**

The objectives of this study were to investigate and comparison of (I) non-enzymatic antioxidants in peels of *C.sinensis, C.reticulata* and *C.aurentifolia* and (II) the free radical scavenging method and NO scavenging method.

Materials And Methods

Chemicals and reagents

All chemicals and reagents were of analytical grade and general purpose reagents unless otherwise stated.

Procurement of samples

SSSThe following fresh citrus fruits a) *C.sinensis* (sweet lime) b) *C.reticulata* (orange) *and* c) *C.aurentifolia* (common lime) were purchased from local market of Allahabad, U.P., India.

Removal of moisture from fruit peels

The fruits were washed thoroughly with tap water and rinsed with distilled water. The fruits were peeled using a sharp knife and the peels were patted dry using filter paper and dried in hot air oven maintained at 55°C ± 2°C. The dried peels were powdered and used for solvent extraction to determine free radicals activity by DPPH free radical scavenging activity method and nitric oxide scavenging activity method.

Extraction method

Extraction of powdered peels was done by the method given by **Kalpna *et al.*, (2011**). Added 5g of dried powder in conical flasks containing different organic solvents *viz.* hexane, acetone, chloroform, methanol and ethanol(50mL) respectively, plugged with cotton and kept on a rotary shaker (120rpm) for 24hrs at 25 ^{0} C. The extract was filtered, supernatant collected and solvent was evaporated on a water bath maintained 5°C below the boiling point of a particular solvent. Reextraction of the residue obtained from each flask was done for 24h. Supernatant was collected, dried and stored at 4°C in air tight bottles.

Estimation of ascorbic acid

The ascorbic acid content was assayed as described by **Omaye *et al*. (1979)**. One gram of fresh material was ground in a pestle and mortar with 5 mL of 10% TCA, the extract was centrifuged at 3500 rpm for 20 minutes. The pellet was re-extracted twice with 10% TCA and supernatant was increased 10 mL and used for estimation. To 0.5 mL of the extract, 1mL of DTC reagent (2,4-Dinitrophenyl hydrazine-Thiourea-$CuSO_4$ reagent) was added and mixed thoroughly. The tubes were incubated at 37°C for 3 hours and to this a solution of 0.75 mL of ice cold 65% H_2SO_4 was added. The tubes were then allowed to stand at 30°C for 30 min. The resulting colour was read at 520 nm in a spectrophotometer. The ascorbic acid content was determined using a standard curve prepared with ascorbic acid and the results were expressed in milligrams per gram fresh weight.

Estimation of carotenoids

Carotenoids were estimated according to the method given by **Jensen (1978)**. Ground 2g of the powdered peels in 20mL of distilled methanol. The solution filtered and the extraction was repeated until the tissue was free from pigments. The filtrates were pooled and partitioned thrice with equal volume of peroxide-free ether using a separatory funnel. Evaporated the combined ether layers at 35°C. Dissolved the residue in ethanol and added 60% aqueous KOH and left overnight. Equal volume of water was added and partitioned twice with ether. Evaporated the ether layers and dissolved the residue in 10ml of ethanol. The absorbance of this solution was read at 450nm. The carotenoids content (mg $100g^{-1}$) in the sample was calculated using a calibration curve prepared using standard high purity β-carotene.

Estimation of flavonoids content

Flavonoids content was estimated according to the method given by **Chang *et al.*, (2002)**.

The reaction mixture (3 mL) consisted potassium acetate, methanol, 1.2% aluminum chloride and sample (1mg mL^{-1}). Incubated at room temperature for 30 min. The absorbance of all the samples read at 415nm. Quercetin was used as positive control. Flavonoids

content was expressed in terms of quercetin equivalent (mg mL^{-1} of extracted compound).

Estimation of total phenols content

Total phenols content was estimated according to the method given by **Mc Donald *et al*., (2001).** Mixed 0.5mL of extract and 0.1mL Folin-Ciocalteau's reagent and the mixture was incubated at RT for 15min. Added 2.5mL saturated sodium carbonate solution and further incubated for 30min.at 25^{0}C and the absorbance read at 760nm.Gallic acid was used as a positive control. Total phenol value was expressed in terms of gallic acid equivalent (mg ml^{-1} of extracted compound).

Determination of Antioxidant Activity

DPPH free radical scavenging method

The DPPH free radical scavenging activity was determined by the method given by **Mc Cuhn &John (2002).**The reaction mixture consisted of DPPH in methanol (0.3mM) and different dilutions (50-1000µg mL^{-1}) of the extracts dissolved in the solvent. The reaction mixture was incubated for 10min in dark, after which the absorbance was measured at 517nm. BHT was used as positive control. IC_{50} value is the concentration of the sample required to scavenge 50% free radicals was calculated.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was determined by the method given by **Balakrishanan *et al.*, (2009).** The reaction mixture contained Sodium nitroprusside (in phosphate buffered saline) mixed with different concentrations of the extracts (100-2000 µg ml^{-1}) prepared in methanol & incubated at 25^{0}C for 30 min. A control without the test compound, but with an equivalent amount of methanol was taken. After 30min., 1.5mL of the incubated solution was removed and diluted with 1.5mL of Griess reagent. Absorbance was measured at 546nm.Gallic acid was used as positive control.

Results And Discussions

The content of ascorbic acid, carotenoids, flavonoids and total

phenolics in peels of three citrus fruits species *viz*. *C.reticulata, C.aurentifolia and C.sinensis*, respectively are summarized in **Table 1**.

Ascorbic acid content

Ascorbic acid content was found to be maximum (36.9 $mg100^{-1}g$ fresh wt.tissue) in *C.sinensis* whereas *C.aurentifolia* contained the least ($8.42mg100^{-1}g$). **Suriyavathana and Subha (2011)** reported that among *Phyllanthus acidus, Phyllanthus emblica and Citrus limon*, the ascorbic acid content was found to be higher in *Citrus limon.*

Carotenoids content

Using the standard curve of β-carotene, the carotenoids content was found to be highest in *C.reticulata* peels (164µg mL^{-1}) while *C.sinensis* had lowest value (15µg mL).

The findings are in agreement with **Pascual *et al*., (1993**) who reported that carotenoids are localized in the peel and the pulp of different citrus fruits, although they are not evenly distributed within both structures. Also, **Melendez-Martinez *et al.,* (2008**) reported that orange juices in general are a source of carotenoids. **Fratianni *et al*., (2010)** reported total carotenoids content of 25.60± 2.69µg mL^{-1} in fresh orange juice.

Flavonoids Content

The total flavonoids content mL^{-1} of extract powder was higher in peels with the range of 0.04-0.083mg quercetin equivalent. *C. aurentifolia* had the highest flavonoid contents (0.083 mg quercetin equivalent) while *C.sinensis* had the lowest value (0.04 mg quercetin equivalent). The increase in flavonoids content over *C.sinensis* was found to be 107.5%. **Ghasemi *et al.,* (2009)** reported 0.3 mg quercetin equivalent/g of extract powder of *C.reticulata* var. page peels and 7.2 mg quercetin equivalent/g of extract powder of *C.sinensis* peels var. Valencia. **Ghafar *et al.*, (2010)** also reported 10.67± 0.27mg/100mL of juice of flavonoid contents in *C.aurentifolia* and 2.99 ± 0.09mg/100mL of juice of flavonoid contents. The results presented in this study are in agreement with these authors.

Total Phenols Content

The total phenolics contents mL $^{-1}$ of extract powder was higher in peels with the range of 12.5-14.94mg gallic acid equivalent (GAE). *C.sinensis* peels showed the highest value (14.94mg GAE). Phenolics are the most wide spread secondary metabolites in the plant kingdom. Phenolic compounds are a class of antioxidant agents, which act as a free radical scavengers. Similar findings are reported by **Xu *et al*., (2008)** who reported total phenolic content of 149.9 (GAE/100mL of juice) from *C.sinensis*. Also, **Ghasemi *et al.* (2009**) found 104.2mgGAE/g of extract powder of phenolic contents in peels of *C.reticulata* var. Page, and 132.9mgGAE/g of extract powder of phenolic contents in peels of *C.sinensis* var.Valencia. **Ghafar *et al.*(2010)** reported total phenol content of 211.70 (GAE/100mL of juice) from *C.aurentifolia* and *C.sinensis* had 135.3 (GAE/100mL of juice**).**

Determination of Antioxidant Activity

DPPH free radical scavenging method

In the present work, three citrus species peels, in ethanol, methanol, chloroform and acetone solvents were evaluated for their free radical scavenging activity using BHT as standard and following results were obtained:

Ethanolic and methanol extracts of *C. reticulata* peels in **Fig.1** show highest scavenging activity (68.3% and 70.7% respectively) at 1000 µgmL $^{-1}$ concentration which were almost near to that of standard BHT.Whereas, chloroform extracts had lowest activity (62.2%). Acetone extract of *C.sinensis* peels in **Fig.2** shows highest scavenging activity (70.5%) while methanol extract had lowest value (56.5%),Whereas methanol extract of *C.aurentifolia* peels in **Fig.3** shows highest scavenging activity (74.4%) while chloroform extract had lowest value (53.9%). These results are in agreement with **Spada *et al*. (2008)** who have reported high DPPH scavenging activity of lemon fruit compared to orange in methanol extract. The IC_{50} values of BHT, EtOH, MtOH, acetone and $CHCl_3$ extracts are

shown in **Fig.1a, Fig.2a** and **Fig.3a** for *C. reticulate, C.sinensis* and *C.aurentifolia* peels respectively**.** The lower IC_{50} value represents the higher antioxidant activity of the tested samples (**Kalpna *et al.* 2011).**

Nitric oxide scavenging activity

The nitric oxide scavenging activity of different extracts of *C.reticulata, C.sinensis* and *C.aurentifolia* peels respectively are represented in **Fig.4, Fig.5 and Fig.6**. The percentage inhibition of nitric oxide generation by these three citrus species were found to be maximum in ethanol extracts (83.6%, 64.3%, and 70.3% respectively) whereas acetone extracts showed minimum percentage inhibition of nitric oxide generation (47.2%, 50.8% and 53.7% respectively) at 2000 μgmL^{-1}. The standard, gallic acid at the same concentration exhibited 98.3% inhibition. Since, literature on nitric oxide scavenging activity in citrus fruits/peels is not readily available therefore, similar literature on other plants/fruits is been quoted in this dissertation. Our results are in agreement with **Kumar *et al.* (2008*)*** who reported that *Citrullus colocynthis* fruit in methanol extract had 61.4 ± 3.8% nitric oxide scavenging activity at 2500 mg mL^{-1} and also with **Balakrishnan *et al.* (2009)** who reported that the ethanol extract of *Acalypha indica* roots had maximum activity of 64.74% at 1000µg mL^{-1}.

The acetone, methanol and ethanol extracts of citrus peels gave good free radical scavenging activity, which were almost near to that of standard BHT. This is in agreement with **Kalpna *et al.*, (2011)** who reported that the more polar solvents appear to be better than non-polar solvents. In least-polar solvent, chloroform extract showed moderate free radical scavenging activity (IC_{50}>400µg/mL).Therefore, it can be concluded that extracting solvents play an important role in expression of antioxidant activity. Ethanol extract of *C.reticulata* peels showed good antioxidant activity as compared to other citrus species extracts. This may be due to high phenol, ascorbic acid contents in it.

Table 1 Non-enzymatic antioxidant levels in 3 different varieties of citrus peels

Citrus species	Common name	Vit C content (mg /100 g fresh wt. tissue)	Total Phenols content *	Flavonoids content **	Carotenoids Content (µg/ml)
C.reticulata	Orange	19.2± 1.9	14.6 ± 1.4	0.079 ± 0.007	164 ± 14.4
C.aurentifolia	Common lime	8.4± 0.8	12.5 ± 1.3	0.083 ± 0.008	55.1 ± 5.0
C.sinensis	Sweet lime	36.9± 3.7	14.9 ± 1.6	0.04 ± 0.001	15.0 ± 1.5

* mg gallic acid equivalent (GAE).

** mg quercetin equivalent

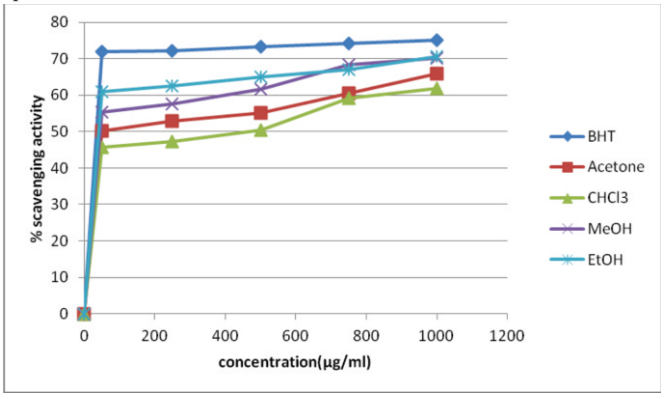

Fig 1: DPPH scavenging activity of *C. reticulata* extracts.

All values are expressed as MEAN of three replicates.

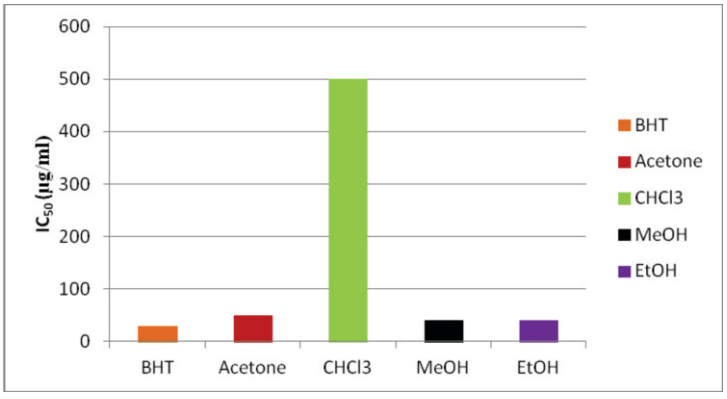

Fig 1a: IC_{50} value in extracts of *C.reticulata*

All values are expressed as MEAN of three replicates.

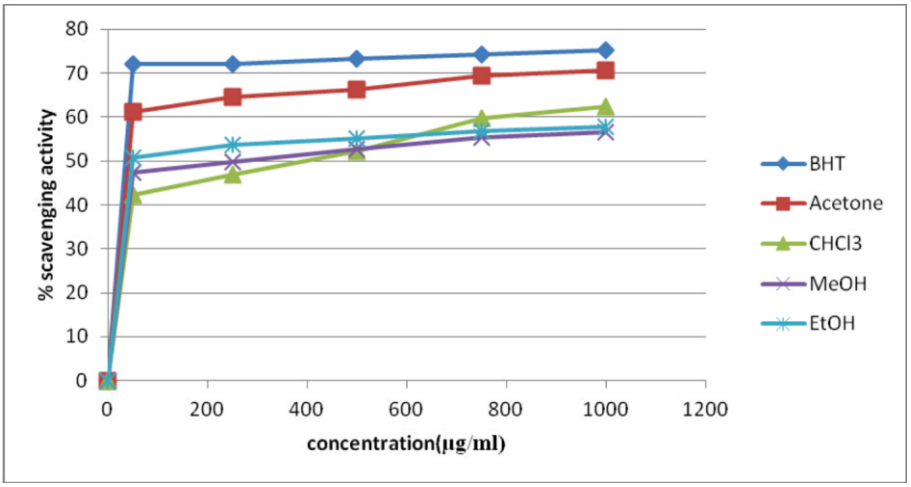

Fig 2: DPPH scavenging activity of *C. sinensis* extracts.

All values are expressed as MEAN of three replicates.

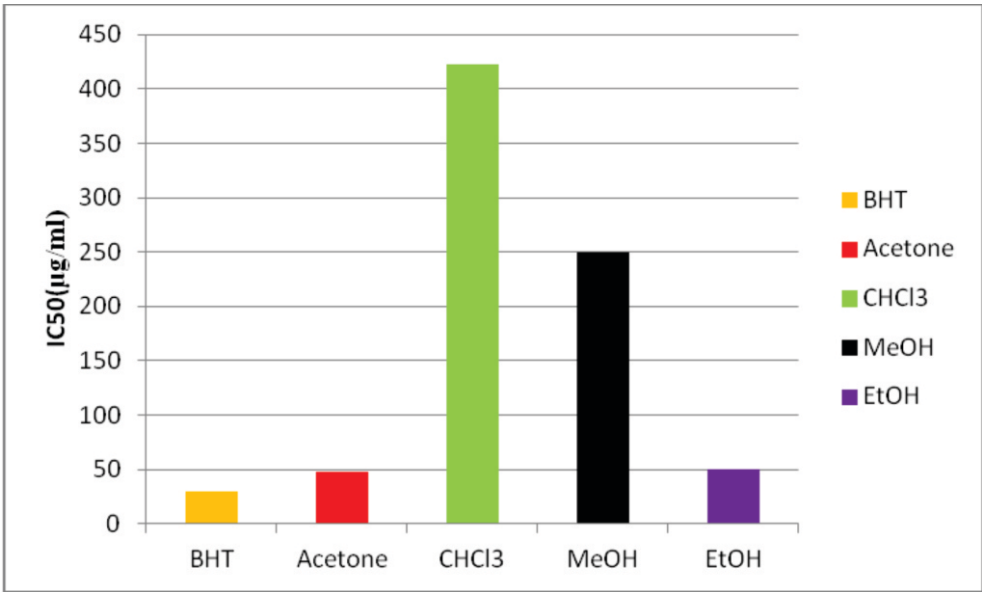

Fig 2.a IC_{50} value of extracts of *C.sinensis*

All values are expressed as MEAN of three replicates.

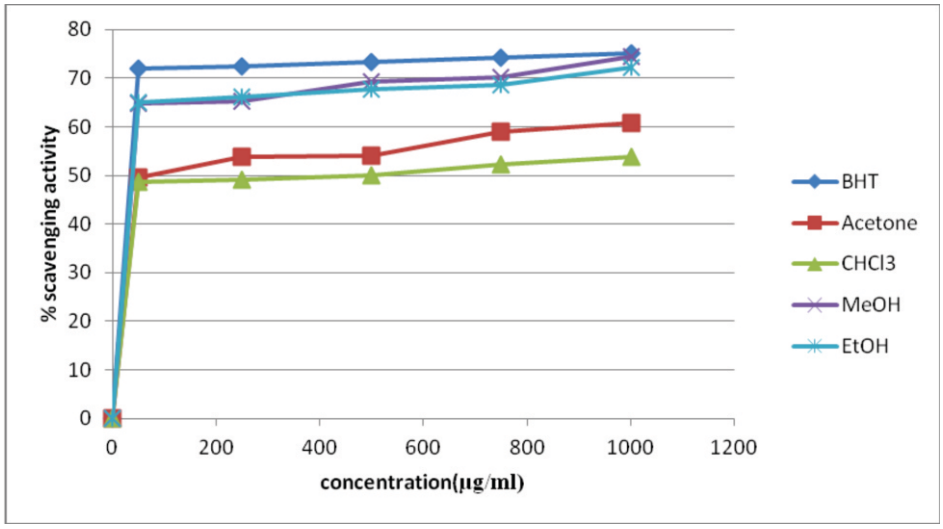

Fig 3: DPPH scavenging activity of *C. aurentifolia* extracts.
All values are expressed as MEAN of three replicates.

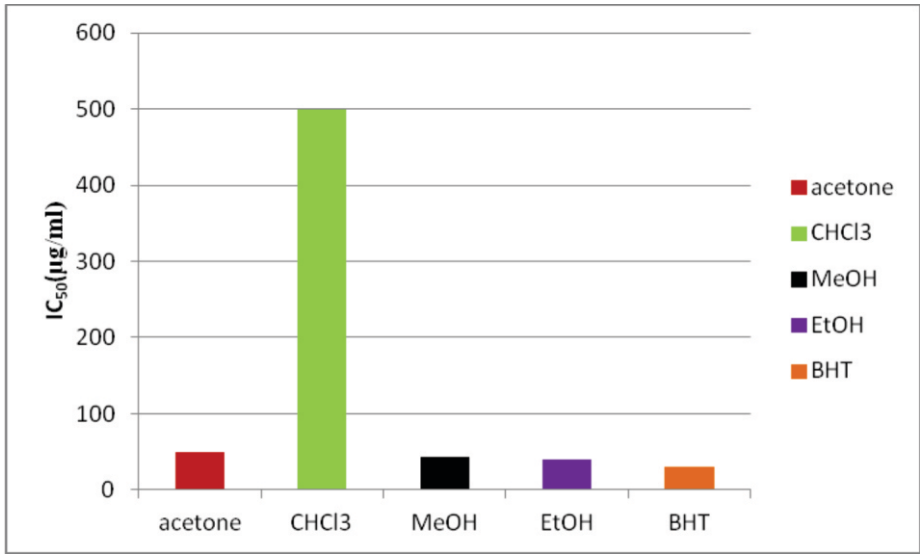

Fig 3.a: IC_{50} value of extracts of *C.aurentifolia*.
All values are expressed as MEAN of three replicates.

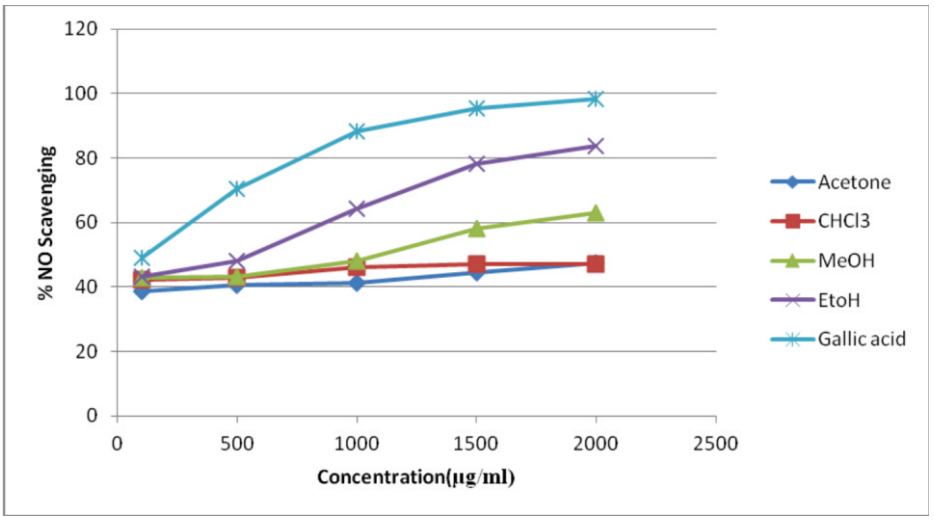

Fig 4: Nitric oxide (NO) scavenging activity of *C.reticulata*
All values are expressed as MEAN of three replicates.

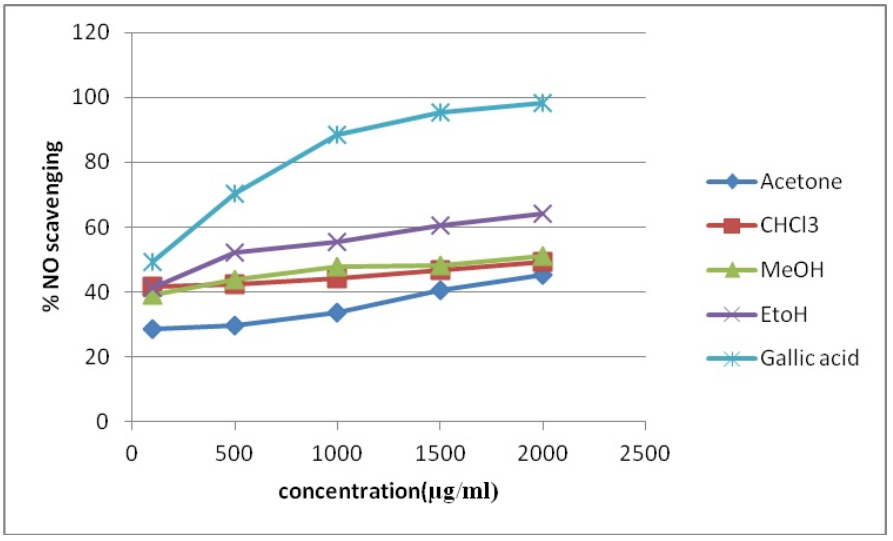

Fig 5: Nitric oxide scavenging activity of *C.sinensis*
All values are expressed as MEAN of three replicates.

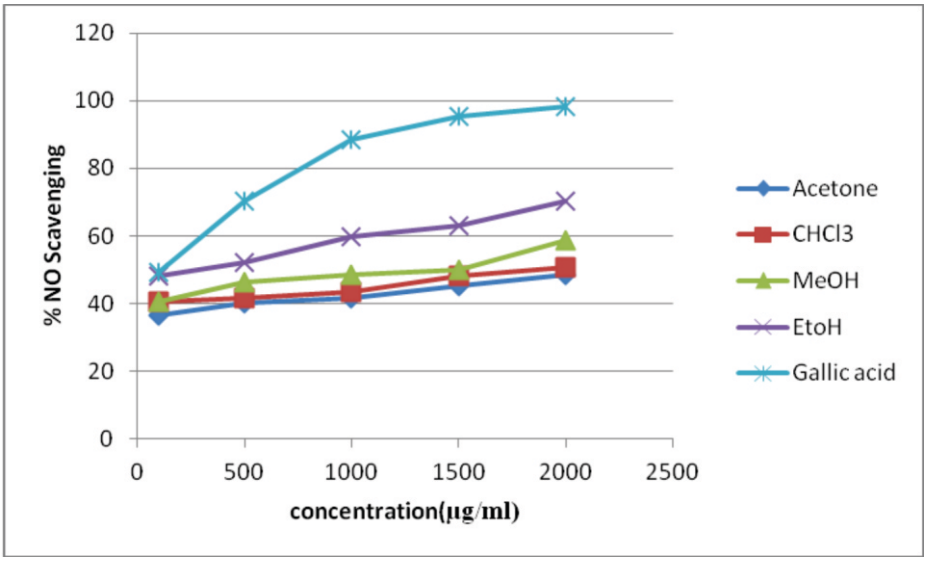

Fig 6: Nitric Oxide Scavenging activity of *C.aurentifolia*
All values are expressed as MEAN of three replicates.

Conclusion

It is widely accepted that consumption of fruits and vegetables is beneficial to health and contributes to decrease of the mortality rate of cardiovascular and other diseases. This study conducted for the determination of non-enzymatic antioxidants *viz.*ascorbic acid, carotenoids, flavonoids and total phenolic contents in three citrus fruits peel. Also, antioxidant activity in acetone, chloroform, methanol and ethanol extracts of three citrus fruits peel by DPPH scavenging activity and nitric oxide scavenging activity was determined. From the results obtained, it is concluded that amongst these 3 citrus fruits peels; the levels of non-enzymatic antioxidants varied amongst these species. Ascorbic acid and total phenols were highest in peels of *C.sinensis* (36.9mg/100g and 14.94 mg gallic acid equivalent respectively), flavonoids content was highest in peels *C. aurentifolia* (0.083mg quercetin equivalent) and carotenoids content was maximum in *C. reticulata* peels (164µg/mL). Antioxidant activity of each extract

increases with concentration. Ethanolic extracts of these citrus fruits peels exhibited maximum antioxidant activity except in peels of *C.sinensis* which showed best activity in acetone extract. This may be due to the fact that the more polar solvents appear to be better than non-polar solvents. The results obtained suggest metabolism of enzymatic antioxidants is more or less similar in citrus fruits peel whereas production of non-enzymatic antioxidants varies. Low IC_{50} values in free radical scavenging activity of the ethanolic extracts indicate a potential for cheap and readily available natural source of antioxidants with health protective potential, which can be used in pharmaceutical, nutraceutical and food preparations. However, further studies required before these can be used as a source of antioxidants.

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