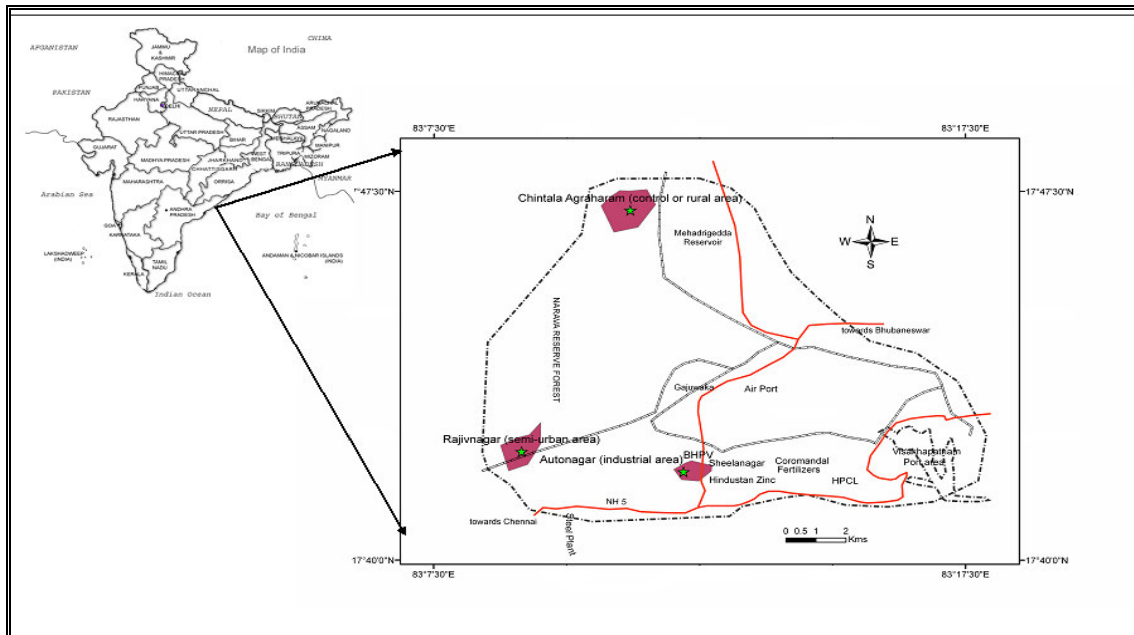


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MANAGEMENT OF INSECT PESTS OF *VIGNA RADIATA* (L.) WILCZEK

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Abstract. Investigation on the evaluation of certain management schedules against major insect pests of *Vigna radiata* (L.) Wilczek, was carried out for two crop seasons (July to October 2001 and 2002) at the Agronomy Farm and the Department of Agricultural Zoology and Entomology of Rajasthan College of Agriculture, Maharana Pratap University of Agriculture & Technology, Udaipur, India. The efficacy of *Azadirachta indica* A. Juss oil and malathion, as first application against aphids, jassids and whiteflies was significantly lower under sole crop of *V. radiata* than when it was inter-cropped with maize during both years (2001 and 2002). Among the different treatment schedules as third application, endosulfan was most effective against the pod borers (*Maruca testulalis* Geyer and *Lampides boeticus* L.) in both sole crop and the intercrop. During the two-year study (2001 and 2002), the maximum yield of maize and green gram in the inter-cropped pattern and that as sole crop of green gram, as well as the maximum rupee equivalent yield value was recorded for the management schedule comprising release of *Chrysoperla carnea* 25 DAS, spray of *A. indica* oil 40 DAS and endosulfan 55 DAS. The lowest yield of *V. radiata* was recorded under the management schedule comprising three release of *Chrysoperla carnea* Stephen at 25, 40 and 55 DAS irrespective of the cropping pattern.

Keywords: Management, insect pests, *Vigna radiata*, *Lampides boeticus*, *Maruca testulalis*

Introduction

Pulses occupy a unique position in the agricultural economy of India being the major source of proteins in Indian dietary. Moreover, their role in improving the fertility of the soil, by microbial fixation of atmospheric nitrogen, further enhances their importance and utility. Traditionally, the farmers grow pulses as companion crops on marginal and unirrigated lands. The area covered by pulses in the country is 14.35 per cent of the total cropped area. *Vigna radiata* (L.) Wilczek is a native of India and Central Asia and has been grown in these regions since prehistoric times (8). It is an important source of easily digestible high quality protein for vegetarians and sick persons and contains 24 per cent protein, 0.326 per cent phosphorus, 0.0073 per cent iron, 0.00039 per cent carotene, 0.0021 per cent of niacin and energy 334 cal/100 g of green gram (4). A number of factors are responsible for its low yield, however, losses caused by insect pests are one of the major factors, therefore, proper and effective pest management methods should be adopted to minimize the losses caused by them. Hence, different management schedules to curb insect pests of *V. radiata* as sole crop and when intercropped with maize were evaluated.

Materials and Methods

Crop cultivation

To evaluate the effect of intercropping green gram with maize as a component in the management of insect pests, sole crop of green gram was sown in plots of size 12m² (4m×3m) maintaining the row-to-row spacing at 30 cm and plant-to-plant distance at 10 cm. In the intercropped system, green gram and maize (1:1 ratio) were sown in plots of 12m² as alternative rows at a distance of 30 cm apart. The experiment was laid out as a factorial RBD. Sole green gram formed one block and the intercrop formed another block. There were 6 treatments in each block accruing to 12 treatments in all that were replicated thrice.

Management of the major insect pests viz., aphids, jassids, white flies and the pod borer complex (*Lampides boeticus* Linnaeus and *Maruca testulalis* Geyer) was manifested using a combination of the botanical (*A. indica* oil), the bio-agent (*Chrysoperla carnea* Stephen) and insecticide (malation and endosulfan treatments. The efficacy of various treatment schedules on these insect pests of green gram, when grown as a sole crop, or when intercropped with maize was studied by observing the reduction in population of the sucking pests and the per cent pod infestation due to the pod borer complex after the first and third application only. The second application comprised release of *C. carnea* at 40 DAS and one spray of *A. indica* oil (0.2%) at 40 DAS and was evaluated as the overall effect on yield and rupee equivalent parameters.

Treatment schedules

T₁ = Spraying of malathion (0.05%) at 25 days after sowing and endosulfan (0.07 per cent) at 55 days after sowing.

T₂ = Three releases of *Chrysoperla carnea* Stephen @ 25000 neonate larvae per hectare 25 DAS, at 40 DAS and at 55 DAS.

T₃ = Three sprays of *Azadirachta indica* A. Juss oil (0.2%) at 25 DAS, at 40 DAS and at 55 DAS.

T₄ = Release of *C. carnea* @ 25000 neonate larvae per hectare at 25 DAS, spraying of *A. indica* oil (0.2%) at 40 DAS and malathion (0.05%) at 55 DAS.

T₅ = Release of *C. carnea* @ 25000 neonate larvae per hectare at 25 DAS, spraying of *A. indica* oil (0.2%) at 40 DAS and endosulfan (0.07%) at 55 DAS.

T₆ = Control

Observations

The population counts were made one day before the treatment application (pre treatment population), and 1, 3, 5 and 7 days after treatment (post treatment population) on five randomly selected plants for the sucking pests aphids, jassids and white flies. The reduction in population observed after the treatments were corrected applying the correction factor as given by Henderson and Tilton (1955). Likewise, for percent damage to pods due to the pod borer/s, ten plants were randomly selected and observations on percent damage to pods were made on these plants, 1, 3, 5 and 7 days after the treatment application.

Estimation of population reduction

$$\text{Reduction in population (\%)} = 100 \times \left\{ 1 - \frac{T_a \times C_b}{T_b \times C_a} \right\}$$

Where,

T_a = Number of insects after treatments

T_b = Number of insects before treatments

C_a = Number of insects in untreated check after treatments

C_b = Number of insects in untreated check before treatments

Statistical analyses

Statistical analyses were done only for comparing efficacy of insecticides and the botanical pesticide (*A. indica* oil) at first and third application. The release of the bio-agent, *C. carnea* was not compared with any of the other treatments for percent population reduction. However, the bio-agent release was compared with respect to percent pod infestation due to the pod borer complex at third application (*i.e.* 55 DAS). Besides, the overall effect of all the treatments after three applications at 25, 40 and 55 days after sowing was also evaluated after computing the rupee equivalent value for the yield obtained under each schedule.

Results and Discussion

The major insect pests recorded were aphids (*Aphis craccivora* Koch.), jassids (*Empoasca kerri* Pruthi and *Empoasca* sp.), whiteflies (*Bemisia tabaci* Genn) and the pod borer complex (*Maruca testulalis* Geyar and *Lampides boeticus* Linn).

Efficacy of management schedules on population reduction of sucking pests

Aphids

After the first application (25 DAS), the aphid population was significantly reduced under sole green gram 1, 3, 5 and 7 days after treatment rather than when inter-cropped with maize during both years (2001 and 2002). From the *Table 1.*, this shows the relative population reduction of insect pests of *Vigna radiata* (L.) Wilczek after pesticide application at 25 DAS, it can be inferred that the maximum reduction in the population of aphids occurred 5 days after treatment in the sole crop (70.36% during 2001), while during 2002 malathion was more effective in the intercrop (52.49%) and *A. indica* in sole crop (59.04%). Malathion was as effective as *A. indica* oil 1, 3, 5 and 7 days after treatment irrespective of whether green gram was sown sole or inter-cropped with maize during 2001; whereas, during 2002 the efficacy of malathion was relatively better when green gram was inter-cropped with maize (*Table 1.*). Comparing the third application of the treatment schedules (55 DAS), it was observed that endosulfan was most effective in both sole green gram and the inter-crop, maize with green gram during both the years (2001 and 2002). *A. indica* oil treatment against aphids had an intermediate efficacy, whereas, malathion was least effective during both seasons [*Table 2.*, showing relative population reduction of insect pests of *Vigna radiata* (L.) Wilczek, after pesticide application at 55 DAS].

Table 1. Relative population reduction of insect pests of *Vigna radiata* (L.) Wilczek after pesticide application at 25 DAS

TREATMENTS	Per cent population reduction of (days after)							
	2001				2002			
Crop Seasons (July to October)								
Days after treatment	1	3	5	7	1	3	5	7
APHIDS:	<i>Vigna radiata</i> , inter-cropped with Maize, <i>Zea mays</i>							
Malathion (0.05%)	50.00 (45.00)	61.46 (51.63)	63.73 (52.73)	58.25 (49.75)	50.90 (45.52)	53.12 (46.79)	52.49 (46.43)	54.63 (47.66)
<i>Azadirachta indica</i> Oil (0.2%)	51.38 (45.79)	55.12 (47.94)	65.84 (54.24)	61.19 (51.47)	38.34 (38.26)	53.83 (47.20)	51.46 (45.84)	46.98 (43.27)
	Sole <i>Vigna radiata</i> (L.) Wilczek							
Malathion (0.05%)	53.31 (46.90)	68.63 (55.94)	70.36 (57.02)	64.43 (53.39)	43.61 (41.33)	43.61 (41.33)	43.61 (41.33)	39.45 (38.91)
<i>Azadirachta indica</i> Oil (0.2%)	53.31 (46.90)	68.63 (55.94)	70.36 (57.02)	64.43 (53.39)	57.83 (49.51)	59.04 (50.21)	59.04 (50.21)	52.56 (46.47)
SEM±	(0.396)	(0.492)	(0.365)	(0.501)	(0.678)	(0.953)	(1.018)	(0.945)
CD at 5% level	(1.370)	(1.705)	(1.263)	(1.733)	(2.345)	(3.297)	(3.522)	(3.269)
JASSIDS:	<i>Vigna radiata</i> , inter-cropped with Maize, <i>Zea mays</i>							
Malathion (0.05%)	41.93 (40.36)	49.91 (44.95)	54.86 (47.79)	50.89 (45.51)	28.99 (32.58)	26.86 (31.22)	30.94 (33.80)	24.05 (29.37)
<i>Azadirachta indica</i> Oil (0.2%)	51.09 (45.63)	59.24 (50.33)	62.40 (52.18)	56.85 (48.94)	39.16 (38.74)	32.93 (35.02)	32.83 (34.96)	24.05 (29.37)
	Sole <i>Vigna radiata</i> (L.) Wilczek							
Malathion (0.05%)	50.97 (45.56)	60.32 (50.96)	58.28 (49.77)	57.75 (49.46)	23.97 (29.32)	35.58 (36.62)	34.58 (36.02)	25.54 (30.36)
<i>Azadirachta indica</i> Oil (0.2%)	50.00 (45.00)	55.53 (48.18)	66.57 (54.68)	52.49 (46.43)	34.69 (36.09)	46.39 (42.93)	49.89 (44.94)	35.29 (36.45)
SEM±	(0.453)	(0.329)	(0.934)	(0.642)	(1.343)	(1.131)	(1.280)	(0.665)
CD at 5% level	(1.567)	(1.138)	(3.231)	(2.225)	(4.646)	(3.913)	(4.428)	(2.300)
WHITE FLIES:	<i>Vigna radiata</i> , intercropped with Maize, <i>Zea mays</i>							
Malathion (0.05%)	42.83 (40.88)	51.55 (45.89)	53.62 (47.08)	47.57 (43.61)	29.93 (33.17)	29.93 (33.17)	30.93 (33.79)	25.68 (30.45)
<i>Azadirachta indica</i> Oil (0.2%)	42.83 (40.88)	61.12 (51.43)	61.65 (51.74)	52.47 (46.42)	29.93 (33.17)	39.72 (39.07)	39.72 (39.07)	46.02 (42.72)
	Sole <i>Vigna radiata</i> (L.) Wilczek							
Malathion (0.05%)	50.00 (45.00)	61.34 (51.56)	57.89 (49.54)	52.89 (46.66)	42.12 (40.47)	44.02 (41.57)	38.78 (38.72)	42.40 (40.63)
<i>Azadirachta indica</i> Oil (0.2%)	50.00 (45.00)	68.98 (56.16)	68.58 (55.91)	60.08 (50.82)	30.12 (33.29)	53.74 (47.15)	53.83 (47.20)	48.13 (43.93)
SEM±	(0.824)	(1.040)	(0.665)	(0.591)	(0.896)	(1.294)	(0.814)	(0.761)
CD at 5% level	(2.851)	(3.598)	(2.300)	(2.044)	(3.100)	(4.477)	(2.816)	(2.633)

1. DAS = Days after sowing.
2. Figures in parentheses are *arc sine* values of per cent reduction.

Table 2. Relative population reduction of inset pests of *Vigna radiata* (L.) Wilczek after pesticide application at 55 DAS

TREATMENTS	Per cent population reduction of (days after)							
	2001				2002			
Crop Seasons (July to October)								
Days after treatment	1	3	5	7	1	3	5	7
APHIDS:								
<i>Vigna radiata</i> , inter-cropped with Maize, <i>Zea mays</i>								
<i>Azadirachta indica</i> Oil (0.2%)	54.47 (47.57)	64.15 (53.22)	65.98 (54.32)	50.90 (45.52)	53.48 (47.00)	54.46 (47.56)	55.97 (48.43)	51.81 (46.04)
Malathion (0.05%)	53.50 (47.10)	63.00 (52.54)	63.53 (52.85)	50.40 (45.23)	53.05 (46.75)	53.24 (46.86)	54.30 (47.47)	49.44 (44.68)
Endosulfan (0.07%)	62.85 (52.45)	71.93 (58.01)	74.11 (59.42)	56.68 (48.84)	58.56 (49.93)	58.45 (49.87)	64.31 (53.32)	65.25 (53.88)
Sole <i>Vigna radiata</i> (L.) Wilczek								
<i>Azadirachta indica</i> Oil (0.2%)	57.63 (49.39)	67.57 (55.29)	68.34 (55.76)	55.90 (48.39)	55.26 (48.02)	56.40 (48.68)	58.81 (50.08)	55.85 (48.36)
Malathion (0.05%)	49.80 (44.89)	55.86 (48.37)	57.14 (49.11)	49.75 (44.86)	53.40 (46.95)	53.34 (46.92)	55.64 (48.24)	51.16 (45.67)
Endosulfan (0.07%)	61.04 (51.38)	70.06 (56.83)	70.84 (57.32)	62.50 (52.24)	63.22 (52.67)	63.74 (52.98)	70.40 (57.04)	66.44 (54.60)
SEm±	(0.752)	(0.951)	(1.305)	(1.920)	(0.347)	(0.578)	(0.524)	(0.793)
CD at 5% level	(2.369)	(2.996)	(4.111)	6.048)	(1.093)	(1.820)	(1.650)	(2.498)
JASSIDS:								
<i>Vigna radiata</i> , inter-cropped with Maize, <i>Zea mays</i>								
<i>Azadirachta indica</i> Oil (0.2%)	50.71 (45.41)	63.19 (52.65)	63.29 (52.71)	53.99 (47.29)	51.16 (45.67)	54.21 (47.42)	64.23 (53.27)	52.37 (46.36)
Malathion (0.05%)	49.77 (44.87)	59.33 (50.38)	59.31 (50.37)	50.73 (45.42)	48.83 (44.33)	54.67 (47.68)	55.03 (47.89)	51.23 (45.71)
Endosulfan (0.07%)	62.01 (51.95)	75.27 (60.18)	76.00 (60.67)	58.02 (49.62)	57.99 (49.60)	62.02 (51.96)	70.79 (57.29)	66.70 (54.76)
Sole <i>Vigna radiata</i> (L.) Wilczek								
<i>Azadirachta indica</i> Oil (0.2%)	57.99 (49.60)	70.46 (57.08)	69.10 (56.23)	57.59 (49.37)	54.04 (47.32)	56.90 (48.97)	65.48 (54.02)	58.30 (49.78)
Malathion (0.05%)	54.18 (47.40)	61.41 (51.60)	60.10 (50.83)	53.05 (46.75)	50.06 (45.04)	55.86 (48.37)	55.24 (48.01)	54.51 (47.59)
Endosulfan (0.07%)	65.20 (53.85)	74.95 (59.97)	75.49 (60.33)	62.19 (52.06)	62.06 (51.98)	62.99 (52.53)	72.12 (58.13)	71.84 (57.95)
SEm±	(0.645)	(0.866)	(0.969)	(1.195)	(0.842)	(0.554)	(1.153)	(0.838)
CD at 5% level	(2.032)	(2.728)	(3.052)	(3.764)	(2.652)	(1.745)	(3.632)	(2.640)
WHITEFLIES:								
<i>Vigna radiata</i> , inter-cropped with Maize, <i>Zea mays</i>								
<i>Azadirachta indica</i> Oil (0.2%)	54.53 (47.65)	54.61 (47.65)	67.59 (55.30)	49.66 (44.81)	51.37 (45.79)	54.63 (47.66)	58.92 (50.14)	53.05 (46.75)
Malathion (0.05%)	48.13 (43.93)	48.13 (43.93)	56.09 (48.50)	41.74 (40.25)	51.81 (46.04)	55.74 (48.30)	56.73 (48.87)	50.69 (45.40)
Endosulfan (0.07%)	57.87 (49.53)	65.26 (53.89)	68.34 (55.76)	57.78 (49.48)	61.99 (51.94)	63.36 (52.75)	64.05 (53.16)	62.02 (51.96)
Sole <i>Vigna radiata</i> (L.) Wilczek								
<i>Azadirachta indica</i> Oil (0.2%)	55.55 (48.19)	61.02 (51.37)	63.64 (52.92)	52.94 (46.69)	52.91 (46.67)	57.83 (49.51)	61.55 (51.68)	57.51 (49.32)
Malathion (0.05%)	53.88 (47.23)	56.64 (48.82)	58.92 (50.14)	54.21 (47.42)	55.83 (48.35)	56.05 (48.48)	57.09 (49.08)	51.60 (45.92)
Endosulfan (0.07%)	63.83 (53.03)	71.82 (57.94)	71.27 (57.59)	58.23 (49.74)	65.61 (54.10)	64.03 (53.15)	74.95 (59.97)	71.46 (57.17)
SEm±	(1.202)	(1.494)	(0.932)	(1.059)	(0.923)	(0.651)	(0.557)	(0.547)
CD at 5% level	(3.786)	(4.706)	(2.936)	(3.336)	(2.907)	(2.050)	(1.754)	(1.723)

DAS = Days after sowing. Figures in parentheses are *arc sine* values of per cent reduction.

Jassids

Following the first application (25 DAS) malathion and endosulfan were significantly more effective in the sole crop 1, 3 and 7 days after application during 2001. However, the efficacy of *A. indica* oil was more pronounced in the inter-crop, while that of malathion was so in sole green gram. In the subsequent year (2002), *A.*

indica oil was superior in the inter-crop 1 day after application, while its efficacy was superior in the management of jassids in sole green gram 3 and 5 days after treatment. Malathion happened to be less effective than *A. indica* oil against jassids in green gram inter-cropped with maize (Table 1). In the subsequent application (55 DAS) endosulfan was more superior to *A. indica* oil and malathion in both sole green gram and green gram inter-cropped with maize during 2001, but in the subsequent year (2002) endosulfan happened to be significantly better in the sole crop. During both seasons *A. indica* oil had an intermediate efficacy irrespective of the cropping pattern. Malathion was least effective during the two-year study under sole as well as the inter-crop (Table 2.).

Whiteflies

At first application (25 DAS), malathion and *A. indica* oil efficacy against whiteflies was significantly more pronounced under the sole crop rather than when inter-cropped with maize during both years (2001 and 2002) (Table 1.). At third application (55 DAS), endosulfan was significantly superior to *A. indica* oil and malathion in sole green gram as well as when green gram was inter-cropped with maize in the first year (2001) of experimentation. In the next year (2002), the efficacy of endosulfan was more pronounced in the sole crop rather than when green gram was inter-cropped with maize. During the first season (2001) *A. indica* oil happened to be more effective than malathion against whiteflies, whereas, in the subsequent year (2002) *A. indica* oil and malathion had a similar effect irrespective of cropping pattern. Foliar application with cypermethrin, deltamethrin and dimethoate 50 days after sowing of green gram proved quite effective in reducing the incidence of *Bemisia tabaci* and the virus (1). The maximum effectiveness was recorded by Borah *et al* (1996) for dimethoate (0.03%) at 15 and 30 days after germination, followed by dimethoate (0.03%) at 15 days after germination + malathion (0.05%) at 30 days after germination. The use of cotton as a trap crop, sown one month ahead between the green gram rows, with a single spray of dimethoate (0.03%) at 15 days after germination of green gram, effectively controlled both *Bemisia tabaci* (11.83/10 plants) and yellow mosaic virus (7.81%).

Efficacy of management schedules (55 DAS) against pod borers

(i) *Lycaenid* pod borer

Endosulfan was most effective against the borer infesting green gram either as sole crop or when inter-cropped with maize recording the lowest pod damage in both the years (2001 and 2002) of study. The efficacy of *A. indica* oil was intermediate, but was more pronounced when green gram was inter-cropped with maize. Malathion happened to be least effective against the borer in the both the years. The release of *Chrysoperla carnea* @ 25000/ha as a third application had an almost equal effect as that of malathion but was better than control [Table 3., showing the comparative efficacy of different treatments on *Lampides boeticus* Linnaeus infesting *Vigna radiata* (L.) Wilczek]. Comparing the efficacy of three synthetic pyrethroids: fenvalerate (0.01%), cypermethrin (0.0075%) for the control of *Euchrysops cnejus* on green gram (*Vigna radiata*) and cowpea (*Vigna unguiculata*) with that of quinalphos (0.05%) and endosulfan (0.07%), it was observed that the performance of synthetic pyrethroids was superior to that of either quinalphos or endosulfan. The residue levels on pods were

generally higher on cowpea than on green gram, but no residue of any compound except endosulfan (0.013 ppm in cowpea and 0.062 ppm in green gram) was detected in the grain (6).

Table 3. Comparative efficacy of different treatments on *Lampides boeticus* Linnaeus infesting *Vigna radiata* (L.) Wilczek

Crop Season (July to October)	2001				2002			
	1	3	5	7	1	3	5	7
Treatments with dose	<i>Vigna radiata</i>, inter-cropped with <i>Karanj</i>, <i>Zea mays</i> L.							
<i>Chrysoperla carnea</i> Stephen (25000/ha.)	11.30 (19.65)	11.30 (19.65)	11.30 (19.65)	13.65 (21.69)	12.65 (20.84)	13.65 (21.69)	13.65 (21.69)	12.98 (21.12)
<i>Azadirachta indica</i> Oil (0.2%)	8.31 (16.76)	8.31 (16.76)	8.31 (16.76)	10.65 (19.05)	9.97 (18.41)	10.32 (18.74)	10.65 (19.05)	11.31 (19.66)
Malathion (0.05%)	10.32 (18.74)	10.65 (19.05)	10.97 (19.35)	13.65 (21.69)	12.65 (20.84)	13.32 (21.41)	13.32 (21.41)	14.32 (22.24)
Endosulfan (0.07%)	5.65 (13.75)	5.64 (13.75)	5.96 (14.14)	8.64 (17.10)	7.32 (15.70)	7.65 (16.06)	7.65 (16.06)	9.97 (18.41)
Control	19.31 (26.07)	19.65 (26.32)	20.65 (27.03)	20.65 (27.03)	18.65 (25.59)	18.99 (25.84)	18.31 (25.34)	18.65 (25.59)
Treatments with dose	<i>Sole Vigna radiata</i> (L.) Wilczek							
<i>Chrysoperla carnea</i> Stephen (25000/ha.)	12.95 (21.10)	12.95 (21.10)	12.95 (21.10)	15.97 (23.56)	14.32 (22.24)	13.65 (21.69)	13.65 (21.69)	12.65 (20.84)
<i>Azadirachta indica</i> oil (0.2%)	8.64 (17.10)	8.97 (17.43)	8.97 (17.43)	11.65 (19.96)	10.32 (18.74)	10.65 (19.05)	11.31 (19.66)	12.65 (20.84)
Malathion (0.05%)	11.31 (19.66)	12.32 (20.55)	12.32 (20.55)	14.65 (22.51)	12.32 (20.55)	13.64 (21.68)	13.64 (21.68)	14.31 (22.23)
Endosulfan (0.07%)	6.65 (14.95)	7.32 (15.70)	7.65 (16.06)	9.65 (18.10)	7.65 (16.06)	8.31 (16.76)	8.31 (16.76)	9.65 (18.10)
Control	24.32 (29.55)	24.32 (29.55)	24.98 (29.99)	25.31 (30.21)	24.65 (29.77)	25.31 (30.21)	25.31 (30.21)	25.65 (30.43)
SEm±	(0.483)	(0.544)	(0.535)	(0.307)	(0.341)	(0.328)	(0.341)	(0.346)
CD at 5% level	(1.434)	(1.616)	(1.589)	(0.912)	(1.013)	(0.974)	(1.013)	(1.027)

Data presented are per cent pod infestation. 2 Figures in parentheses are *arc sine* values.

(ii) *Spotted pod borer*

Endosulfan was most effective against spotted pod borer recording lowest pod infestation when green gram was inter-cropped with maize during both the years (2001 and 2002). *A. indica* oil was the next best treatment after endosulfan. During the two crop seasons, malathion as well as release of *Chrysoperla carnea* as third application were least effective against the spotted pod borer [Table 4., showing the comparative efficacy of different treatments on *Maruca testulalis* Geyer infesting *Vigna radiata* (L.) Wilczek].

Soil application of aldicarb at 1.0 kg a.i./ha combined with a spray of endosulfan (0.07%) was more effective in reducing the incidence of yellow mosaic disease and

damage by *Maruca testulalis* Geyer than a combination of endosulfan with soil applications of phorate or disulfoton both at 1.0 kg a.i./ha. However, disulfoton with endosulfan gave the highest increases in crop yield over the untreated variant 32.5 and 19.6 per cent for green gram (*Pusa Baisaki*) and black gram (T-9), respectively (7). In another field trial conducted under dry farming conditions in Tamil Nadu, India, during 1976-77 with green gram (*Vigna radiata*) Var. *Pusa Baisaki*, Sundararaju and Rangrajan (1987) observed that a reduced incidence of yellow mosaic disease (transmitted by the aleyrodid *Bemisia tabaci*) and damage by pod borers (mainly the pyralid, *Maruca testulalis*) were obtained with a basal application of disulfoton at 1.0 kg a.i./ha and a spray of endosulfan (0.07%), applied both at the time of flowering and 15 days later, in combination with fertilizer treatment at 25 kg N and 50 kg P₂O₅/ha. Yield of *V. radiata* increased by 48.7 per cent when both treatments were given simultaneously compared with 31.3 per cent with insecticides alone and 16.8 per cent with fertilizers alone.

Table 4. Comparative efficacies of different treatments on *Maruca testulalis* Geyer infesting *Vigna radiata* (L.) Wilczek

Crop Season (July to October)	2001				2002			
	1	3	5	7	1	3	5	7
Days after treatment								
Treatments with dose	<i>Vigna radiata</i> , intercropped with <i>Zea mays</i> L.							
<i>Chrysoperla carnea</i> Stephen (25000/ha.)	13.32 (21.41)	13.32 (21.41)	12.65 (20.84)	12.32 (20.55)	14.65 (22.51)	14.65 (22.51)	14.32 (22.24)	13.99 (21.97)
<i>Azadirachta indica</i> Oil (0.2%)	11.31 (19.66)	11.31 (19.66)	11.31 (19.66)	11.65 (19.96)	10.32 (18.74)	10.32 (18.74)	10.65 (19.05)	12.32 (20.55)
Malathion (0.05%)	12.32 (20.55)	12.65 (20.84)	12.65 (20.84)	12.65 (20.84)	13.32 (21.41)	13.65 (21.69)	14.32 (22.24)	15.31 (23.04)
Endosulfan (0.07%)	8.64 (17.10)	8.64 (17.10)	8.64 (17.10)	8.97 (17.43)	7.65 (16.06)	7.99 (16.42)	8.31 (16.76)	9.65 (18.10)
Control	22.31 (28.19)	22.65 (28.42)	23.65 (29.10)	23.99 (29.33)	20.31 (26.79)	20.31 (26.79)	20.65 (27.03)	21.99 (27.97)
Treatments with dose	<i>Sole Vigna radiata</i> (L.) Wilczek							
<i>Chrysoperla carnea</i> Stephen (25000/ha.)	16.32 (23.83)	15.64 (23.30)	15.64 (23.30)	14.65 (22.51)	15.31 (23.04)	14.97 (22.77)	14.97 (22.77)	14.65 (22.51)
<i>Azadirachta indica</i> oil (0.2%)	11.97 (20.25)	12.32 (20.55)	12.32 (20.55)	13.32 (21.41)	12.32 (20.55)	12.32 (20.55)	12.32 (20.55)	13.98 (21.96)
Malathion (0.05%)	15.31 (23.04)	15.31 (23.04)	15.31 (23.04)	16.32 (23.83)	14.32 (22.24)	14.65 (22.51)	14.65 (22.51)	15.64 (23.30)
Endosulfan (0.07%)	8.97 (17.43)	9.31 (17.77)	9.65 (18.10)	10.65 (19.05)	9.65 (18.10)	10.32 (18.74)	10.65 (19.05)	12.32 (20.55)
Control	27.64 (31.72)	27.64 (31.72)	27.98 (31.94)	29.31 (32.78)	29.66 (33.00)	29.32 (32.79)	29.66 (33.00)	30.33 (33.42)
SEm±	(0.360)	(0.291)	(0.282)	(0.321)	(0.288)	(0.290)	(0.331)	(0.282)
CD at 5% level	(1.069)	(0.864)	(0.837)	(0.954)	(0.855)	(2.101)	(0.983)	(0.837)

Data presented are per cent pod infestation. Figures in parentheses are arc sine values.

Table 5. Comparative effect of different insect pest management schedules on the yield of *Vigna radiata* and *Zea mays*

Schedule No.	Treatment schedule			Yield as kg./ 12m ² (as q/ha) in the inter crop				Yield as kg./ 12m ² (as q/ha) in sole crop	
				Greengram		Maize		Greengram	
	I (25 DAS)	II (40 DAS)	III (55 DAS)	2001	2002	2001	2002	2001	2002
1.	Malathion (0.05%)	No treatment	Endosulfan (0.07%)	0.16 (1.33)	0.17 (1.42)	5.72 (47.66)	5.96 (49.66)	0.65 (5.42)	0.68 (5.66)
2.	<i>C. carnea</i> (25000/ha.)	<i>C. carnea</i> (25000/ha.)	<i>C. carnea</i> (25000/ha.)	0.12 (1.00)	0.16 (1.33)	5.31 (44.25)	5.31 (44.25)	0.57 (5.75)	0.65 (5.42)
3.	<i>A. indica</i> Oil (0.2%)	<i>A. indica</i> Oil (0.2%)	<i>A. indica</i> Oil (0.2%)	0.25 (2.08)	0.23 (1.92)	6.54 (54.50)	6.94 (57.83)	0.76 (6.33)	0.83 (6.92)
4.	<i>C. carnea</i> (25000/ha.)	<i>A. indica</i> Oil (0.2%)	Malathion (0.05%)	0.14 (1.16)	0.19 (1.58)	4.75 (39.58)	4.98 (41.50)	0.65 (5.42)	0.75 (6.25)
5.	<i>C. carnea</i> (25000/ha.)	<i>A. indica</i> Oil (0.2%)	Endosulfan (0.07%)	0.34 (2.83)	0.30 (2.50)	6.96 (58.00)	7.61 (63.42)	0.89 (7.42)	0.99 (8.25)
6.	Control	Control	Control	0.09 (0.75)	0.10 (0.83)	4.02 (33.50)	4.10 (34.16)	0.42 (3.50)	0.51 (4.25)
	S. Em. ± CD at 5% level			0.002	0.007	0.03	0.006	0.010	0.017
				0.007	0.024	0.09	0.021	0.032	0.054

DAS=Days after sowing

Effect of management schedules on grain yield

During both the seasons (2001 and 2002) the maximum yield of maize and green gram in the inter-cropped pattern and that of green gram in sole crop was recorded for the management schedule comprising release of *Chrysoperla carnea* 25 DAS, *A. indica* oil 40 DAS and endosulfan 55 DAS; whereas, the management schedule comprising *Chrysoperla carnea* 25 DAS, *A. indica* oil 40 DAS and malathion 55 DAS recorded the lowest yield in inter-cropping. The lowest yield of green gram was recorded under management schedule comprising three releases of *Chrysoperla carnea* at 25, 40 and 55 DAS. An overall observation showed that the net returns from inter-cropping were significantly higher than that of sole green gram, which is a clear case of cropping pattern advantage [Table 5., depicting the comparative effect of different insect pest management schedules on the yield of *Vigna radiata* (L.) and *Zea mays* L.].

In an earlier study on nitrogen utilization efficiency, as affected by component populations in maize/ green gram inter-cropping, conducted at Los Banos, Philippines in 1988 dry season, maize (cv. IPB Var. 2) and green gram cv. Pag-asa (5) were grown in pure stands at their optimum plant populations of 50000 and 300000 plants/ha, respectively, or were inter-cropped in combinations of maize populations of 25000, 50000 or 75000/ha and green gram populations of 150000, 300000 or 450000/ha. Maize grain yield was 3.82 t/ha in the pure stand and 2.16 to 2.55 t/ha when inter-cropped. Yield was not significantly affected by green gram plant density in the inter-crops, but was highest at a maize plant density of 50000/ha. Green gram seed yield was 1.57 t/ha in pure stand and 0.69 to 0.80 t in inter-crops. In the inter-crops, green gram yield was highest at the lowest maize plant density and 300000 green gram plants/ha. Inter-cropping reduced nitrogen absorption by both crops. The proportional reduction in absorption efficiency of maize ranged from 0.28 to 0.37 and that of green grams from 0.50 to 0.57. Increasing the maize population increased the N absorption efficiency of maize, but decreased the efficiency of green grams. The N absorption efficiency of all inter-

crops was higher than that of the sole crops added together. Land equivalent ratio (LER) analysis showed that the LER was highest when both components of the inter-crop were at their optimum sole crop population, and that the yield advantage was primarily due to an increase in N absorption efficiency in the inter-crops (3).

Effect of management schedules on rupee equivalent value

Of the various management schedules compared for the rupee equivalent value obtained due to sole green gram as well as green gram inter-cropped with maize, the maximum rupee equivalent yield value during both years (2001 and 2002) of study was recorded for the management schedule comprising *Chrysoperla carnea* 25 DAS, *A. indica* oil 40 DAS and endosulfan 55 DAS (Rs. 39, 691 per hectare and Rs. 46, 291 per hectare). The next best treatment schedule comprised of three applications of *A. indica* oil at 25, 40 and 55 DAS (Table 6., showing the comparative rupee equivalent values for yield under different management schedules applied to *Vigna radiata* (L.) Wilczek, as sole crop and when intercropped with *Zea mays* L.).

Table 6. Comparative rupee equivalent values for yield under different management schedules applied to *Vigna radiata* (L.) Wilczek as sole crop and when intercropped with *Zea mays* L.

Schedule No.	Treatment schedule			Rupee equivalent of yield per plot	
	I (25-DAS)	II (40-DAS)	III (55-DAS)	2001	2002
Vigna radiata intercropped with Zea mays					
T ₁	Malathion (0.05%)	No treatment	Endosulfan (0.07%)	37.05 (30875.00)	41.54 (34616.66)
T ₂	<i>C. carnea</i> (25000/ha.)	<i>C. carnea</i> (25000/ha.)	<i>C. carnea</i> (25000/ha.)	33.94 (28283.33)	37.23 (31025.00)
T ₃	<i>A. indica</i> Oil (0.2%)	<i>A. indica</i> Oil (0.2%)	<i>A. indica</i> Oil (0.2%)	43.76 (36466.66)	48.88 (40733.33)
T ₄	<i>C. carnea</i> (25000/ha.)	<i>A. indica</i> Oil (0.2%)	Malathion (0.05%)	30.93 (25775.00)	35.62 (29683.33)
T ₅	<i>C. carnea</i> (25000/ha.)	<i>A. indica</i> Oil (0.2%)	Endosulfan (0.07%)	47.63 (39691.66)	55.55 (46291.66)
T ₆	Control	Control	Control	25.77 (21475.00)	28.31 (23591.66)
Vigna radiata as sole crop					
T ₁	Malathion (0.05%)	No treatment at	Endosulfan (0.07%)	11.10 (9250.00)	12.30 (10250.00)
T ₂	<i>C. carnea</i> (25000/ha.)	<i>C. carnea</i> (25000/ha.)	<i>C. carnea</i> (25000/ha.)	9.68 (8066.66)	11.85 (9875.00)
T ₃	<i>A. indica</i> Oil (0.2%)	<i>A. indica</i> Oil (0.2%)	<i>A. indica</i> Oil (0.2%)	13.03 (10858.33)	15.00 (12500.00)
T ₄	<i>C. carnea</i> Stephen (25000/ha.)	<i>A. indica</i> Oil (0.2%)	Malathion (0.05%)	11.10 (9250.00)	13.35 (11125.00)
T ₅	<i>C. carnea</i> Stephen (25000/ha.)	<i>A. indica</i> Oil (0.2%)	Endosulfan (0.07%)	15.18 (12650.00)	17.85 (14875.00)
T ₆	Control	Control	Control	7.25 (6041.66)	9.30 (7750.00)
S. Em. ± CD at 5% level				0.119	0.237
				0.348	0.695

N.B.: Rate 2001 2002
 Maize = Rs. per quintal 600/- 645/-
 Greengram = Rs. per quintal 1750/- 1800/-
 DAS = Days after sowing

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EVALUATION OF TOTAL HYDROCARBON LEVELS IN SOME AQUATIC MEDIA IN AN OIL POLLUTED MANGROVE WETLAND IN THE NIGER DELTA

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Abstract. The total hydrocarbon (THC) levels in the surface waters, sediments and biota in an oil polluted mangrove wetland located in the Niger Delta Nigeria, were studied between November 2001 and October 2002. Result showed elevated THC mean levels in water ($23.6 \pm 4.3\text{mg/l}$), sediment ($386.44 \pm 50.28\mu\text{g/g}$), *Tympanotonus fuscatus* (Periwinkle) ($449.30 \pm 55.42\mu\text{g/g}$) and *Periophthalmus papillio* (Mudskipper) ($278.57 \pm 34.57\mu\text{g/g}$), indicating a polluted environment. THC levels in water at the wellhead stations were above 10mg/l being the maximum recommended limit by the Federal Ministry of Environment (FMENV) Nigeria. There was a pronounced seasonal variability with the highest being in the dry season ($P < 0.05$) due to the higher level of increased oil activity in that season. THC levels in water correlated significantly ($r = 0.927$) with THC levels in the sediment ($P < 0.01$). The implication is that as the THC remains within the surface water column in relation to the sediment the more the organisms in the environment are impacted. This is shown by the significant correlations ($P < 0.01$) of THC between the organisms and their surrounding media. The high levels of THC in the surface water and sediments of these stations suggest that aquatic life water quality may adversely influence biological functions of exposed species, while the level in the *Tympanotonus fuscatus* and the *Periophthalmus papillio* calls for concern as it can have some health-risk implications in man who is the final consumer.

Key words: *Molluscs, biota, bioaccumulation, hydrocarbon*

Introduction

The extraction and usage of petroleum products as energy sources the world over has led to a widespread pollution of the biosphere. About 6-10 million barrels of crude oil enter the aquatic environment yearly [1]. The control of such pollution problems in the aquatic environment is very difficult because of the large number of input sources and their geographic dispersions. Contrary to popular views, evidence is accumulating to buttress the fact that petroleum hydrocarbon mixes with water and penetrates to the underlying sediments [2, 3, 4]. The resultant effects of the above are a change in desirable portable water characteristics [5]; impaired growth of marine organisms which depend basically on the quantity and quality of the primary production of phytoplankton, fish, crustaceans and molluscs acquire objectionable odour or flavour, thereby causing a reduction in their marketability and acceptance as food [6, 7]. Death of the fauna and flora from oil spills is common place in the Niger Delta region of

Nigeria where the most intensive oil exploration, exploitation and refining occur [8]. Besides, oil contamination of coastal amenities has adverse effects on tourism, recreation and aesthetics of the impacted area. This effect can be substantial on a community whose economy depends on tourism.

Mudskipper (*Periophthalmus papillio*) and Periwinkles (*Tympanotonus fuscatus* var. *radula*) are of economic importance to the natives in the Niger Delta. Periwinkles are rich in protein (21.04%) and carbohydrates and are gathered daily for food [9]. Their nutritional values compare favourably with those of domestic livestock and fish [7]. Like all other intertidal organisms mudskipper and periwinkles are very vulnerable to oil pollution because their habit are susceptible to coating with oil and may be smothered in the event of heavy oil drifting ashore. The creek and its environs bearing the flow station facilities play important role in the economic well being of the natives of the area as they ferment cassava into raw 'fufu' (locally made starchy food) from dug out holes on the mudflats of the area and fishing operations that is being carried out on a daily basis.

The aim of this research was to provide information on the total hydrocarbon status of the surface waters, sediments and biota (periwinkle and mudskipper) of the area.

Study area

The area is a mangrove wetland with lots of creeks and creeklets that links up to the lower reaches of the New Calablar River. The creeks are characterized by high seawater inflow and low freshwater input from runoffs of domestic wastewater, adjoining farmlands and forest and intense oil exploration and exploitation activities. Five sampling stations were chosen within the study area as indicated in *Fig.1*.

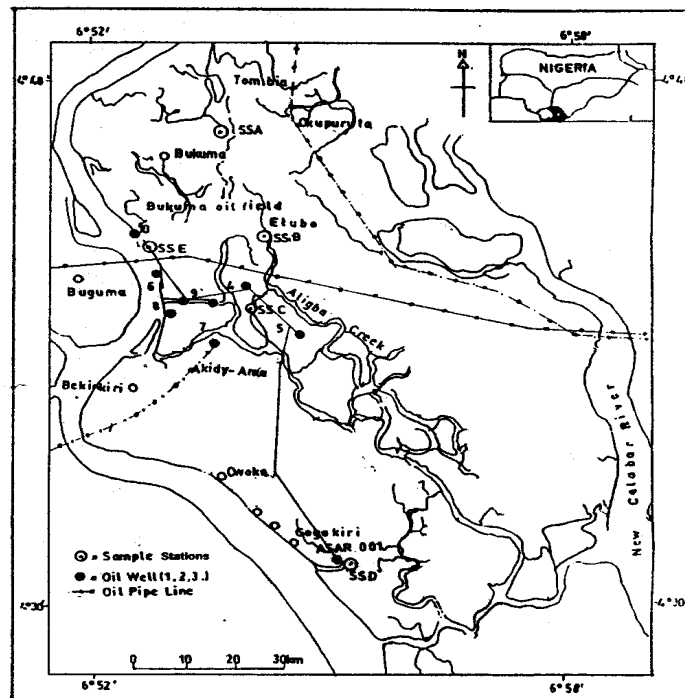


Figure 1. Map of the study area showing sampling stations

Station A (SSA- reference) was an area without any industrial activities, except local fishing, periwinkle picking, etc. Station B (SSB) was an extension of the main creek (wherein the flow station was located) that leads to farmlands and where fermentation of cassava on the mangrove mudflats of the area was carried out. Station C (SSC) was the flow station that bears crude oil production facilities. There was also a nearby oil wellhead. Station D (SSD) was along a navigational route for barges and outboard and inboard engine boats. It had an oil wellhead. Station E (SSE) was a highly disturbed area by a recent oil and gas blow out from the oil wellhead. Most of the mangrove trees were destroyed. There was also an intense surface water runoff from the adjoining farmlands and forest.

Materials and Methods

Water and sediment samples were collected by the method of [10], while periwinkles (3.0 ± 0.5 cm mean length) and mudskippers (3.5 ± 0.5 cm mean length) were collected according to [7 and 11], respectively. Each sample was concurrently collected in triplicates from each station bi-monthly between November, 2001 and October, 2002.

In the laboratory (Institute of Pollution Studies Rivers State University of Science and Technology Port Harcourt) the sediment samples were air dried for four days, while the periwinkle and fish samples were frozen. Frozen samples were later thawed and the whole muscle tissues of the periwinkles were chucked out from the shells. The muscle tissues of the mudskippers were dissected out. Samples were dried on aluminum foil by heating in an oven at 50°C for 48hours to constant weight. Heat treatment had the advantage of preserving the tissue for longer period. Besides it simulates the process they undergo before consumption in the Niger Delta area [7].

Tissue samples were ground in a teflon mortar, while the sediment samples were macerated and then sieved through a 1 μ m sieve for total hydrocarbon (THC) determination. 5.0g each of the sieved samples were later extracted with two 25.0ml portions of toluene. The sieved samples were shaken on a Stuart flask shaker for 10 mins. The extracts were later filtered into 50.0ml flasks and made up to the 50.0ml mark with toluene. Total hydrocarbon from the water samples were extracted with 30ml toluene for three consecutive times and later made up to 100ml. The absorbances of the filtrates were measured spectrophotometrically at 420nm with a Spectrophotometer 21D [12 and 13]. The concentration calculated from the calibration graph on dry weight basis. Appropriate blanks were run throughout the procedure.

Data were analysed by a multivariate analysis of variance and means reported by Duncan's Multiple Range Test (DMRT). The THC periwinkles and fish tissues were correlated with that in surface water and sediment [14]. The level of significance was set at 95% and 99%, respectively.

Results

The total hydrocarbon content of all the media differed in the sampling period and sites (*Table 1.; Figs. 2. and 3.*). The trend of concentrations from all the media deferred significantly ($P < 0.05$), thus periwinkle > sediment > mudskipper > surface water, while that of the stations was SSE > SSC > SSD > SSB > SSA (*Fig. 4.*) and the seasonal variation was: dry > wet season (*Fig. 5.*), except in the mudskipper muscles. In all the months of the study (November 2001 - October 2002) the levels of THC were higher ($P < 0.05$) in

the periwinkle tissues than the other media (Fig. 4.). The monthly trend of all the metal levels in all the media within the study period was March=Jan>May=Nov>July>November= September (Fig. 3.).

Table 1. Mean values of the total hydrocarbon content of four media from the study area in comparison with other related studies

STNs of this study/Other Studies	Surface waters (mg/l)	Sediment (µg/g)	Periwinkle tissues (µg/g)	Mudskipper Fish Muscles (µg/g)	STNs TOTAL MEAN/Other References
A	4.07 ± 1.44 ^c	112.31 ± 17.96 ^c	134.61 ± 9.26 ^c	121.51 ± 14.75 ^c	93.13 ± 49.61 ^e
B	6.51 ± 2.06 ^c	190.49 ± 15.13 ^d	194.28 ± 25.00 ^c	208.73 ± 30.54 ^{cd}	150.00 ± 85.21 ^d
C	40.66 ± 3.79 ^a	514.11 ± 71.14 ^{ab}	664.35 ± 78.81 ^{ab}	381.21 ± 46.40 ^a	400.08 ± 155.63 ^b
D	21.05 ± 5.35 ^b	457.98 ± 56.67 ^c	541.15 ± 76.64 ^{ab}	266.36 ± 46.41 ^c	321.64 ± 108.74 ^c
E	45.71 ± 8.86 ^a	657.31 ± 95.14 ^a	712.10 ± 83.37 ^{ab}	415.06 ± 34.80 ^a	457.55 ± 169.45 ^a
TOTAL MEAN	23.60 ± 4.30 ^d	386.44 ± 50.28 ^b	449.30 ± 55.42 ^a	278.57 ± 34.57 ^c	

Within column (Stations), mean ± sem with different superscript are significantly different at P<0.05
For the total mean (media), mean ± sem with different superscript are significantly different at P<0.05
Stations total mean ± sem with different superscript are significantly different at P<0.05

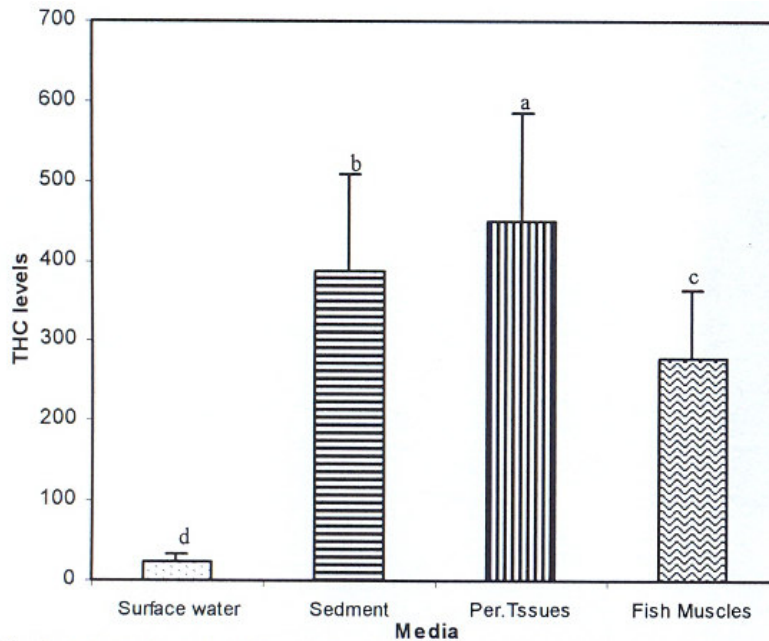


Figure 2. Mean levels of THC in the surface water (mg/l), Sediment (µg/g), Periwinkle tissues (µg/g) and Mudskipper fish muscles (µg/g) from the study area. Note: Superscripts with different alphabets are significantly different (P<0.05)

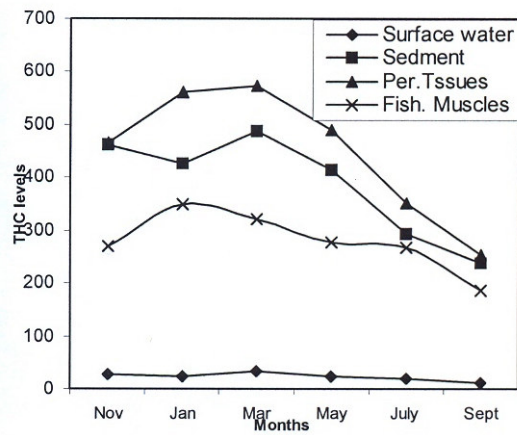


Figure 3. Monthly variations of THC in the Sediments ($\mu\text{g/g}$) and surface water (mg/l) Periwinkle tissues ($\mu\text{g/g}$) and Mudskipper fish muscles ($\mu\text{g/g}$) from the study area.

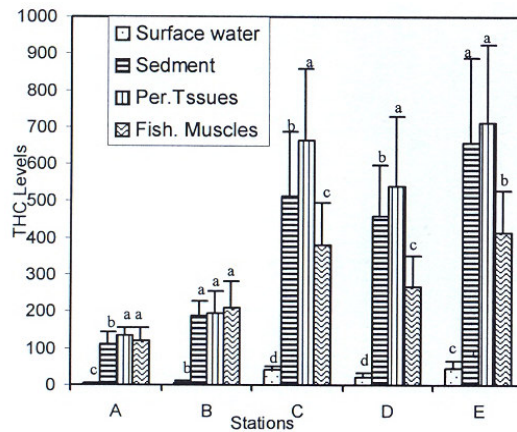


Figure 4. Stations variations of THC in the surface water (mg/l), Sediment ($\mu\text{g/g}$), Periwinkle tissues ($\mu\text{g/g}$) and Mudskipper fish muscles ($\mu\text{g/g}$) from the study area. Note: Superscripts with different alphabets are significantly different ($P < 0.05$)

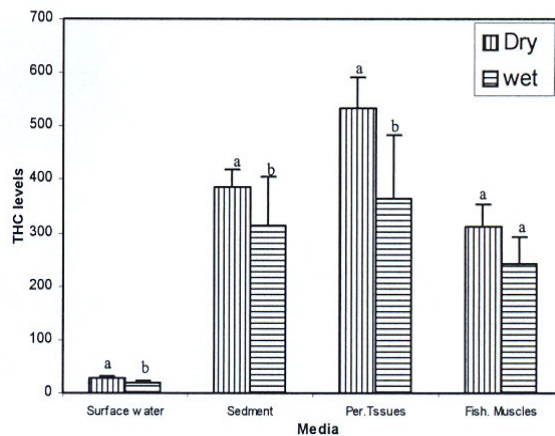
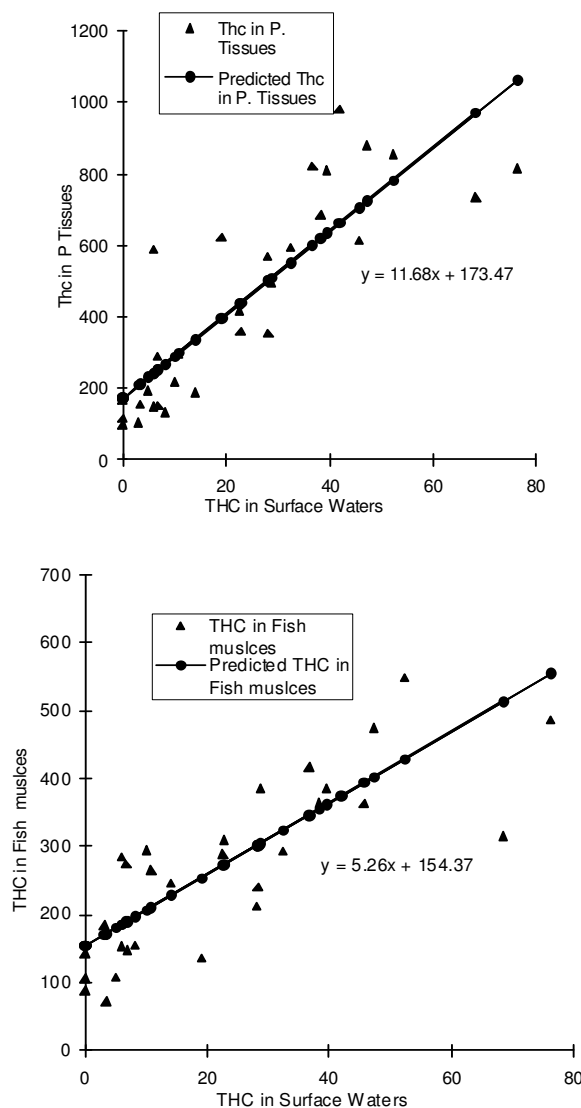


Figure 5. Seasonal variation of THC in the surface water (mg/l), Sediment ($\mu\text{g/g}$), Periwinkle tissues ($\mu\text{g/g}$) and Mudskipper fish muscles ($\mu\text{g/g}$) from the study area. Note: Superscripts with different alphabets are significantly different ($P < 0.05$)

Linear relationship analyses in the levels of THC between the biota (periwinkles and mudskippers) and their exposure media - surface water and sediment were all significant ($P < 0.01_{0.46}$). The various bioaccumulation and correlation factors are as shown in Table 2. and Fig. 6.

Table 2. Bioaccumulation factors and correlation coefficients of the periwinkles and mudskippers to surface water and sediment

Organism	Exposure media	K_b	r
Periwinkle tissues	Surface water	11.69	0.74
Periwinkle tissues	Sediment	1.06	0.92
Mudskipper muscles	Surface water	5.26	0.79
Mudskipper muscles	Sediment	0.48	0.87



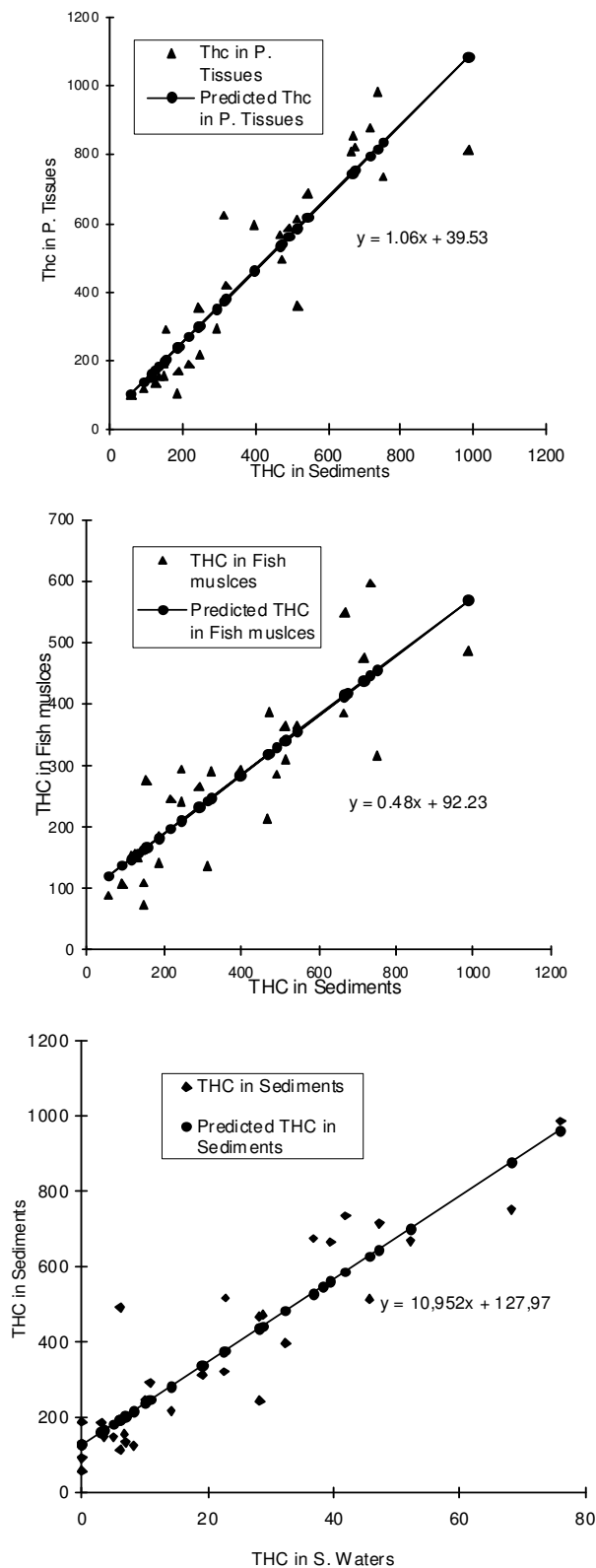


Figure 6. Relationship of the THC levels between the biota and the exposure media (Sediment and Surface water) of this study

Discussion

Generally, higher total hydrocarbon concentrations were recorded in stations that have oil formations than those without oil formations. However, the high recorded in SSB, without formation, may imply input result from other sources like domestic wastes, discharge of sewage, drifts from polluted areas and other activities [15]. The level of THC contamination decreased significantly ($P < 0.05$) between stations with wellheads and those without, possibly due to dilution effects which may have gradually lowered the level of total hydrocarbons from the inception of the sampling period (November 2001) when there was increase of oil activities within the study area to the end of the sampling period (October 2002). In addition, various chemical, physical and biological processes that are known to degrade petroleum hydrocarbon in water may undoubtedly have contributed to the general decrease in the hydrocarbon levels observed. THC levels in the dry season were higher than in the rainy season for the stations with wellheads contrary to the observations of some authors [16, 11 and 17]. However, in the non-oil formation stations (SSA and SSB), higher levels were recorded in the rainy season than in the dry season. The oil must have been washed into the river from land based sources during the rainy season in these stations, besides increased water current and wave action, which may largely disturb the sediment with the concomitant resurfacing of the previously leached hydrocarbon into the sediment [17]. It has been remarked that high temperatures and high rates of microbial activities are known to cause rapid degradation of petroleum hydrocarbons released into tropical environment [16].

The regression analysis indicates that the biota accumulated THC from their immediate environment. For instance from the regression equation, a unit increase of THC in the surface water of the study area will add 11.6 and 5.6 units of THC into the periwinkle tissues and mudskipper muscles respectively. This is also shown in the respective bioaccumulation factors. By implication both organisms (though the periwinkles were better off) were good accumulators and indicators of pollutants in effluents and other wastes in the river system. Again the sediment loads of THC all through the sampling periods and sites were consistently higher than that of the surface water indicating that it was a better indicator of pollution in the river system even after the sources of pollution has been removed.

High hydrocarbon content causes oxygen deterioration by reduction in gaseous diffusion through the surface film of oil with far reaching implications for the flora and fauna of the affected area [18]. The presence of oil on the mangrove floor as was observed in sample stations C and E for instance will encumber the decay process of the litters from mangroves, which ordinarily enhance the accumulation of organic matter. Also, oiling of the mangrove system may also lead to leaf loss or complete defoliation of the characteristic *Rhizophora* and *Avicennia* ssp mangrove seedlings in the affected areas [19] as was observed in station E. Shriveling, occupying-up of leaves, arrested expansion of buds and some degrees of foliar necrosis have also been identified as a mark of stress of a mangrove system plagued with petroleum hydrocarbons [12]. In a related field reconnaissance survey an abysmal decimation of juvenile red mangrove by oil spillage was observed along the Nembe axis of Niger Delta resulting from either short or long time exposure of the flora to toxic effects of the petroleum hydrocarbons [18]. It has also been noted that the growth of organisms that depend basically on primary producers (phytoplanktons and algae) and zooplanktons were always adversely affected under such conditions [6 and 20].

In the light of the forgoing the high mean surface water and sediment load of THC for stations C and E suggest that normal functioning of feeding, respiration and movement of benthic organisms may be adversely affected. Besides the levels in the *T. fuscatus* and *P. papillio* can have some health-risk implications in man who is the final consumer. The national permissible limit of total hydrocarbon/oil grease for inland water is 10mg/l [21], while the WHO permissible level of THC in seafood is 0.001µg/g [22]. This indicates that the study area was generally contaminated with petroleum hydrocarbon and the continuous consumption of seafood from the area may pose a public health hazard. However, the THC levels in the study area, was similar to that in other studies in the Niger Delta by [11]: surface water - 42.05mg/l and sediment - 184.89µg/g; [20] surface water - 149mg/l, sediment - 339.2µg/g and biota - 196.9µg/g and [23] sediment - 528.25µg/g.

The variation in the pattern of the total hydrocarbon within the study area suggests that most of the hydrocarbons in sediment, water and biota were of anthropogenic origin. The lower levels in the reference station A and at least station B suggest that these were the concentrations generally present in this area and anthropogenic source may have added to the aquatic media burden of total hydrocarbon in the environment. Moreover the FMENV's maximum discharge limit of 10mg/l THC/oil and grease into inland waters is less than the mean value in this study. This finding corroborates that of the World Bank [24] report that of all the 5,500 tons of hazardous wastes produced per year in Rivers State, the petroleum Industry, including the refineries generates most of them. There is therefore the need to develop management plan to ensure that petroleum hydrocarbon contamination of the area is prevented in order to achieve good aquatic life water quality and avoid any possible negative health out-break through consumption of contaminated marine resources by the local communities.

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ENVIRONMENTAL VARIATION IN SEED AND SEEDLING CHARACTERISTICS OF *PINUS ROXBURGHII* SARG. FROM UTTARAKHAND, INDIA

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Abstract. The present study was undertaken to assess the environmental variation in seed characters and to explore the efficacy of hydrogen peroxide treatment on the germination and seedling traits of sixteen provenances of *Pinus roxburghii* from Uttarakhand Himalaya. Provenances, which had higher values for seed parameters showed better germination. Soaking the seeds for 24 hours in a solution of H₂O₂ (1% v/v) had a significant effect on the rate of germination and average germination percentage. About 88.5 % of mean average germination was revealed by the seeds that were treated with H₂O₂ as compared to the untreated (control) seeds (77.4 %). Simultaneously, H₂O₂ treatment also caused an appreciable decrease, in shortening of germination period by 10 days. Results have shown that the soaking of chir-pine seeds in H₂O₂ (1% v/v) for 24 hours is highly beneficial and therefore for large scale germination, treatment of H₂O₂ should be preferred. The germination percentage of seeds was found to be positively correlated with altitude and negatively correlated with rainfall.

Keywords: *Garhwal Himalaya, Provenance, Altitude, Hydrogen per oxide treatment, Seedling height.*

Introduction

The natural regeneration of different plant species through seeds depends upon the production and germination capacity of the seeds and the successful establishment of the seedlings. Seeds from healthy, well formed trees provide greater assurance that the resulting stock will have good form, survival and resistance against stress conditions. A tree species may extend over the large geographical area and can grow in wide range of environments with varying climate and topography. It usually has local populations adapted to the different environment at conditions, known as provenances. After knowing the range of adaptation of a species its populations may become more important in forest restoration, as global warming and other environmental changes disrupt natural forests (Ledig & Kitzmiller, 1992). In provenance trials, provenances from varying climatic conditions are compared to assess the seed quality. As an early step towards improvement of tree species, provenance trials provide prerequisite knowledge of both genetic and environmental variations, which allow direct genetic comparisons among seed sources growing in multiple “common gardens” and are ideally suited to quantify impacts of changing climate on wood production (Kitzmiller, 2005).

Pinus roxburghii Sargent, commonly known as ‘Chir pine’ is the most important pine among the six indigenous pine species of India, which is much valued for its timber and oleoresin. It occurs in the monsoon belt of the outer Himalaya, from North – eastern part of Pakistan to Arunachal Pradesh in India at elevations varying from 450 to 2300m asl. It is found distributed over a long strip of about 3,200 km between latitudes 26°N to 36°N and longitudes 71°E to 93°E. The species is economically very important

and is used for variety of purposes viz., timber for house construction, fuel wood extraction, charcoal formation, growing trees stems for resin tapping, needles for fuel briquetting, cattle bedding and manufacturing organic manure, etc.

Work on seed testing of various provenances of *Pinus roxburghii* from Uttarakhand and Himachal Himalaya has been done by Sharma et al. (Sharma et al., 2001), Ghildiyal et al. (2007), and Ghildiyal and Sharma (2005, 2007), whereas, Isoenzyme analysis on different provenances of Chir pine from the Indian subcontinent has been done by Hussain (1995) from Pakistan, and by Sharma (1999) and Sharma (2007) from India. The germination capacity of seeds is strongly influenced by abiotic factors such as temperature, water stress and, in certain cases, light, which often show a significant influence on germination (Knipe, 1973; Rao & Singh, 1985). Several growth hormones and chemicals like H₂O₂ have been found to enhance the rate of seed germination in many species viz., in spruce (Chandra & Chauhan, 1976), *Northofagus obliqua* and *N. procera* (Shafiq, 1980), *Pinus wallichiana* (Thapliyal et al., 1985), V, *Pinus roxburghii* (Ghildiyal, 2003) etc, through which losses in seed germination could be minimised (Quarberg & Jahns, 2000). In this study we have tried to assess the variation in seed characters and an effort was made to explore the efficacy of hydrogen peroxide treatment on the germination and seedling characters of 16 provenances of *Pinus roxburghii* collected from Garhwal Himalaya.

Materials and methods

The study was conducted on the seed and seedling characteristics of 16 provenances of *Pinus roxburghii* collected from different geographic locations, which were distributed in 4 districts i.e., Pauri, Chamoli, Rudraprayag and Tehri of Uttarakhand state in India (latitude 29° 26' to 31° 28' N and longitude 77° 49' to 80° 06'E). The majority of rain fall (1000mm to 1800mm) in these regions occur during monsoon period i.e., from June to September, and are represented by sub-tropical to temperate climates. The detailed geographical and meteorological attributes of various provenances are given in *Table 1.* & *Fig.1.*

Table 1. Geographical and meteorological descriptions of different provenances of *Pinus roxburghii*.

Provenance	District	Latitude (N)	Longitude (E)	Altitude (m)	Temperature		Mean annual rainfall (mm)
					Min.	Max.	
Ashtavakra	Pauri	30° 13'	78° 48'	960	5.76	37.70	705.00
Agustmuni	Rudraprayag	30° 23'	79° 02'	875	4.31	36.59	833.00
Badiyargarh	Tehri	30° 17'	78° 50'	1080	7.50	36.30	930.00
Ghansali	Tehri	30° 27'	78° 39'	890	5.00	34.60	1230.00
Godnar	Chamoli	30° 30'	79° 16'	1680	1.30	24.00	1890.00
Jaiharikhal	Pauri	29° 47'	78° 32'	960	7.54	37.00	1150.00
Jasholi	Rudraprayag	30° 16'	79° 04'	1520	1.60	34.10	1025.00
Kalimath	Chamoli	30° 34'	79° 05'	1540	1.60	26.10	1257.50
Lansdowne	Pauri	29° 50'	78° 41'	1703	-0.90	25.80	1260.00
Mayali	Tehri	30° 23'	78° 47'	1400	2.60	25.10	1030.00
Pabo	Pauri	30° 15'	79° 01'	1640	1.8	32.4	875.00
Pauri	Pauri	30° 09'	78° 48'	1660	-0.48	26.30	1792.00
Pokhal	Tehri	30° 25'	78° 59'	820	5.70	37.63	800.00
Tangni	Chamoli	30° 29'	79° 28'	1480	4.20	25.50	990.00
Thalisain	Pauri	30° 02'	79° 03'	1640	1.9	31.00	1025.00
Vana	Chamoli	30° 38'	79° 05'	1610	1.30	24.00	1660.00

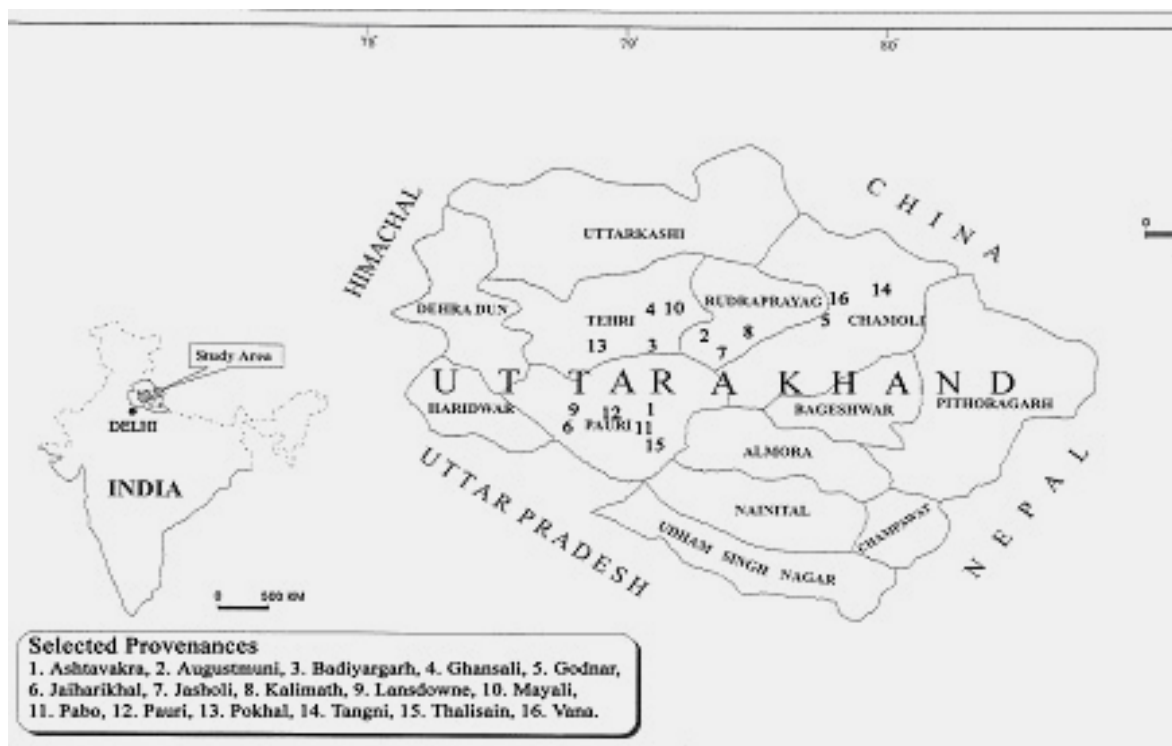


Figure 1. Location map of the study areas

The cones were collected from plus trees which were tallest, straightest, best shaped with well developed crowns and were free from pests and diseases. Ten ripe cones per tree were collected at random from the selected plus trees for recording the seeds length, width and thickness of randomly selected seeds (8 replicates of 20 seeds each) for each seed source. Seed weight of 800 seeds of each seed source (8 replicates of 100 seeds each) was recorded in order to verify the variation in seed weight. The seeds of different provenances having same level of ripeness were collected and subjected to viability test by floating method to select only the viable seeds. Seed germination in all the provenances was carried out under laboratory conditions at 25°C temperature, inside a seed germinator (Model No. 8LT-SGL CALTAN) after applying following presowing treatments to each set:

Treatment 1- Soaking of the seeds in distilled water at room temperature (25°C) for 24 hours.

Treatment 2- Soaking of the seeds in Hydrogen peroxide (H₂O₂ 1% v/v) at room temperature (25°C) for 24 hours.

Observations were recorded daily regarding germinated /non-germinated seeds up to 21 days. Radical emergence was taken as the criteria for germinability. The collected data were further quantified in terms of percent germination and germination value. Percent germination was the value of seeds germinated at the completion of the germination period, whereas, germination value is an index, combining speed and completeness of germination; which according to Czabator (1962) can be expressed as: $G\bar{V} = P\bar{V} \times M\bar{D}G$, where, $G\bar{V}$ is germination value, $P\bar{V}$ is the peak value of germination, and $M\bar{D}G$ is the mean daily germination. The critical difference (CD) was calculated as: $CD = SEd \times t_{0.01}$, Where, SEd is the standard error of difference calculated as $SEd = \sqrt{2Me/r}$, where Me = mean sum of square and r = number of

replicates. The variation in growth and biomass production in different provenances was recorded by transferring the laboratory germinated seedlings to polythene bags containing potting mixture of sand, soil and FYM in the ratio of 1:1:1. The polythene bags containing seedlings were placed in randomized block design, with three replications of each provenance in the homogenous net house conditions. Initially, watering was done daily up to four weeks and thereafter, at 3 days interval, till the commencement of the rainy season. The data on height and root length were recorded at the end of four months.

Results and Discussion

The results obtained on seed characteristics are presented in *Table 2*.

Table 2. Seed characteristics, Germination percentage and Seedling growth in different provenances of *Pinus roxburghii* from Garhwal Himalaya.

Provenances	Seeds weight with wings (gm)	Seed Length (mm)	Seed Width (mm)	Seed Thickness (mm)
Pokhal	13.36 ±0.40	9.34 ±0.23	5.28 ±0.17	3.33 ±0.18
Godnar	11.02 ±0.53	8.25 ±0.20	4.72 ±0.28	2.82 ±0.14
Kalimath	14.00 ±0.51	9.42 ±0.33	5.35 ±0.21	3.61 ±0.21
Agustmuni	11.96 ±0.48	9.14 ±0.31	4.80 ±0.25	3.02 ±0.17
Jasholi	13.26 ±0.65	10.12 ±0.25	5.65 ±0.27	3.85 ±0.23
Tangni	15.02 ±0.55	10.80 ±0.27	5.80 ±0.21	4.23 ±0.20
Vana	13.76 ±0.59	10.65 ±0.21	5.84 ±0.27	4.12 ±0.18
Ashtavakra	12.66 ±0.38	10.08 ±0.27	5.35 ±0.22	3.86 ±0.15
Mayali	11.89 ±0.47	9.36 ±0.30	5.08 ±0.15	3.65 ±0.24
Jaiharikhal	12.78 ±0.60	9.40 ±0.35	5.56 ±0.23	3.23 ±0.20
Lansdowne	13.98 ±0.54	10.28 ±0.29	6.35 ±0.28	4.07 ±0.17
Pauri	14.27 ±0.42	9.99 ±0.20	5.51 ±0.21	3.49 ±0.19
Ghansali	14.88 ±0.49	10.56 ±0.29	5.89 ±0.26	4.01 ±0.14
Thalisain	15.67 ±0.37	10.98 ±0.24	6.98 ±0.18	4.40 ±0.21
Pabo	15.48 ±0.64	10.30 ±0.27	5.84 ±0.15	3.94 ±0.16
Badiyargarh	12.78 ±0.58	8.48 ±0.32	5.36 ±0.23	3.44 ±0.25
F-test	3.890*	0.0614	0.266	2.132*
P-value	0.0006	1.00	0.9956	0.0355
LSD	1.8174	0.8576	0.6126	0.5922
SD	0.803	0.559	0.447	1.339
CV%	8.176	10.006	12.117	9.881

*significant at 5% level.

**ArcSine values are given in parenthesis

Table 2. cont.

Provenances	Germination percentage (control)**	Germination value (control)	Germination percentage (H ₂ O ₂ 1% v/v)**	Germination value (H ₂ O ₂ 1% v/v)
Pokhal	82.2 ±2.06 (65.05)	10.88 ±1.18	88.4 ±0.75 (70.09)	18.29 ±1.61
Godnar	84.0 ±7.42 (66.42)	7.88 ±2.08	80.4 ±1.17 (63.72)	13.24 ±1.21
Kalimath	97.6 ±1.17 (81.09)	6.05 ±1.54	98.4 ±0.75 (82.73)	7.64 ±0.80
Agustmuni	75.2 ±1.02 (60.13)	18.39 ±2.57	83.2 ±1.02 (65.80)	14.97 ±0.94
Jasholi	73.2 ±1.86 (58.82)	6.35 ±0.79	80.0 ±1.42 (63.44)	6.10 ±0.81
Tangni	38.0 ±1.67 (38.06)	3.25 ±1.31	84.0 ±1.48 (66.42)	17.68 ±1.03
Vana	89.6 ±1.72 (71.19)	16.1 ±1.59	95.2 ±1.02 (77.34)	36.94 ±5.10
Ashtavakra	92.4 ±1.47 (74.00)	28.9 ±0.99	94.0 ±1.10 (75.82)	69.97 ±5.74
Mayali	81.6 ±1.17 (64.60)	24.43 ±1.99	88.0 ±0.90 (69.73)	66.62 ±2.87
Jaiharikhal	71.2 ±1.02 (57.54)	4.14 ±1.37	85.2 ±1.02 (67.37)	19.48 ±1.74
Lansdowne	79.6 ±1.60 (63.15)	3.61 ±0.73	92.4 ±0.75 (74.00)	25.64 ±1.59
Pauri	85.6 ±1.17 (67.70)	4.81 ±1.65	90.4 ±1.17 (71.95)	32.19 ±2.35
Ghansali	74.8 ±2.06 (59.87)	3.94 ±0.89	91.2 ±1.10 (72.74)	37.33 ±1.96
Thalisain	90.0 ±1.42 (71.56)	7.26 ±0.86	96.0 ±0.90 (78.46)	43.57 ±1.95
Pabo	84.0 ±2.42 (66.42)	5.49 ±0.94	85.2 ±1.02 (67.37)	24.38 ±1.69
Badiyargarh	39.2 ±1.36 (38.76)	2.84 ±0.74	83.6 ±1.33 (66.11)	17.85 ±2.33
F-test	48.6175*	45.049*	9.341*	212.436*
P-value	1E-17	3.15E-17	8.02E-08	1.17E-27
LSD	20.6650	10.2917	6.9224	23.4626
SD	16.773	8.036	5.702	18.896
CV%	21.674	83.321	6.445	66.905

*significant at 5% level.

**ArcSine values are given in parenthesis

Weight of 100 seeds in various provenances/seed sources ranged from 11.02 ±0.53g (Godnar) to 15.67 ±0.37g (Thalisain). The mean weight of 100 seeds in Pabo (15.48 ±0.64g), Tangni (15.02 ±0.55g), Ghansali (14.88 ±0.49g) and Pauri (14.27 ±0.42g) seed sources were close to the maximum seed weight. The maximum seed length was observed (10.98 ±0.24mm) for Thalisain seed source and minimum (8.25 ±0.20mm) for Godnar seed source. The existing variation for seed width was low as compared to seed length and thickness. The seed width varied between 4.72mm (Godnar) to 6.98mm (Thalisain), similarly, the maximum seed thickness (4.40 ±0.21mm) was revealed by Thalisain seed source, however, the thinnest seeds were produced by Godnar seed source (2.82 ±0.14mm).

Germination of seeds in various seed sources after pre-soaking treatment (H₂O₂ 1%v/v) under 25°C temperature has yielded significant differences in seed germination. The maximum germination percentage (98.4 ±0.75%) was recorded for Kalimath seed source, and minimum (80.0 ±1.42%) for Jasholi seed source. However, the higher germination value (69.97 ±5.74) was recorded for Ashtavakra, and lower (6.10 ±0.81) for Jasholi seed source. Under control conditions the highest germination percentage (97.6 ±1.17%) was recorded for Kalimath seed source and lowest (38.0 ±1.67%) for Tangni seed source. Similarly, the highest germination value (28.09 ±0.99) was recorded for Ashtavakra, whereas, lowest germination value for Badiyargarh seed source (2.84 ±0.74).

The performance of seedlings raised from Pabo seed source was superior, having maximum height (10.16 ±0.37cm), and minimum seedling height (8.70 ±0.29cm) was recorded for Agustmuni seed source. The highest root length was recorded for the

seedlings of Pabo seed source (14.62 ± 0.53 cm), and lowest (12.38 ± 0.63 cm) for Mayali seed source (Table 2.).

Simple correlation was calculated between seed characteristics, germination characteristics and geographic parameters viz., altitude, mean annual rainfall (mm), longitude and latitude, etc., results of which are presented in Table 3.

Table 3. Simple correlation coefficients between seed characteristics and geographic variation.

Characters	1	2	3	4	5	6
Seed length (mm)	1.000					
Seed width (mm)	0.797*	1.000				
Seed thickness (mm)	0.904*	0.840*	1.000			
100-Seed weight (gm)	0.811*	0.812*	0.783*	1.000		
Germination % (control)	0.124	0.048	-0.005	-0.007	1.000	
Germination value	-0.074	-0.379	-0.086	-0.479	0.394**	1.000
Germination % (H ₂ O ₂ 1%v/v)	0.464**	0.470**	0.499**	0.429**	0.579**	0.189
Germination value	0.302**	0.152	0.354**	-0.007	0.314**	0.695*
Seedling height (cm)	0.290	0.228	0.410**	0.476**	-0.169	-0.280
Root length (cm)	-0.066	-0.015	0.016	0.264	-0.062	-0.487
Altitude (m)	0.198	0.331**	0.338**	0.108	0.103	0.119
Mean annual rainfall (mm)	-0.074	-0.135	-0.035	-0.122	-0.309	0.014
Latitude N	-0.182	0.031	-0.196	-0.219	0.215	0.046
Longitude E	0.247	0.550**	0.377**	0.247	0.290	-0.113

Significant at 1% level & ** significant at 5% level

Characters	7	8	9	10	11	12
Seed length (mm)						
Seed width (mm)						
Seed thickness (mm)						
100-Seed weight (gm)						
Germination % (control)						
Germination value						
Germination % (H ₂ O ₂ 1%v/v)	1.000					
Germination value	0.447**	1.000				
Seedling height (cm)	-0.121	-0.174	1.000			
Root length (cm)	-0.129	-0.494	0.789*	1.000		
Altitude (m)	0.119	0.371**	0.185	0.016	1.000	
Mean annual rainfall (mm)	-0.340	-0.023	0.079	-0.020	0.556	1.000
Latitude N	0.076	-0.091	-0.289	-0.286	-0.047	0.093
Longitude E	0.199	0.103	0.202	0.049	0.396**	0.090

Significant at 1% level & ** significant at 5% level

Statistically negative correlation was observed between germination value and seed characteristics. A significant positive correlation was observed between seed germination (H₂O₂ 1% v/v) and other seed parameters. Latitude and longitude were found to be significantly and positively correlated with germination of chirpine seeds under normal (control) conditions (0.215 and 0.290) and when treated with H₂O₂ (0.076 and 0.199). Germination value of the H₂O₂ treated seeds showed negative correlation

with latitude, and positive correlation with longitude, whereas, seeds under controlled conditions showed positive correlation with latitude and negative correlation with longitude. Correlation between germination percentage and rainfall was negative in all the seeds treated and germination under controlled conditions, whereas significantly positive correlation was observed between rainfall and altitude ($r = 0.556$). Except for seedling height and germination value of seeds grown under controlled conditions all the other parameters of seed characteristics and germination characteristics were found to be negatively correlated with rainfall. H_2O_2 treatment showed significant effect on seed germination percent in all the seed sources except Godnar seed source (*Table 2*).

It is apparent from the results that seed sources, which had higher values for seed parameters, also showed better performance in germination. These findings have been supported by the concept of Baldwin (1942) and Dunlap and Barnett (1983), according to which, seed size and weight have pronounced effects on seed germination. Generally, large seeds have fast and uniform germination, due to more endosperm nutrient pool (Kandya, 1978). Therefore, seed source variation in germination percent and related traits may be ascribed to the differences in seed dimensions and weight. Germination values varied considerably among seed sources and exhibited a random pattern. Germination value is an index of combining speed and completeness of germination, which itself is a function of seed size and weight (Czabator, 1962; Dunlap & Barnett, 1983). On the other hand, variation observed in time taken to complete germination could be attributed to the differences in germination rate and germination value of the selected provenances. Significant variation in germination values among seed sources is in conformity with those found in fir and spruce by Singh and Singh (Singh & Virendra Singh, 1981).

Soaking the seeds for 24 hours in a solution of H_2O_2 (1% v/v) had a significant effect on the rate of germination and average germination percentage. About 88.5 % of mean average germination was revealed by the seeds that were treated with H_2O_2 , while the mean average germination percentage of untreated (control) seeds was just 77.4 % (*Table 2*). Simultaneously, H_2O_2 treatment also caused an appreciable decrease, in shortening of germination period by 10 days. Similar results were recorded by Chandra and Chauhan (1976) in *Picea smithiana* and Shafiq (1980) in *Nothofagus obliqua* and *Nothofagus procera* seeds. Thus, soaking of chir-pine seeds in H_2O_2 (1% v/v) for 24 hours is highly beneficial and therefore for large scale germination, treatment of H_2O_2 should be preferred, since it also involves the saving of expenditure as compared to other hormones. The cost involved in case of H_2O_2 will be approximately half of the cost of other hormones. It was also evident from the results that although the H_2O_2 improved the germination of seeds in all the sources, still there were variations in its effect on rate of the germination in the provenances, which may be due to variation in geographical locations and the altitude of the seed sources (Holm, 1994; Barnett, 1997; Vera, 1997). Differences in the rate of germination in the provenances have also been documented by Webb and Farmer (1968), Wilcox (1968) and Tewari et al. (2001). It is clear from the results that the sexual reproductive efficiency, which can be assessed by determining germination capacity may vary with altitude. Thus, altitudinal provenances of a species may differ not only in seed germination but also in their reproductive efficiency.

The seed sources varied significantly among themselves with respect to field survival and growth parameters (*Table 2.*) during all stages of measurements. The seedlings of different provenances, when grown under common nursery environmental conditions,

often displayed different patterns of shoot growth (Dormling, 1979; Rehfeldt & Wycoff, 1981). However, it is not always easy to show that such differences are adaptive, presumably in response to the environment of the parent seed origin. Analysis of variation in growth-related traits of several tree species indicated that some portion of the total variation might be under strong genetic control, which is of adaptive importance and leads to the differentiation into distinct populations. In the present investigation, since seeds of all seed sources were raised under common nursery conditions, the environmental influences being reduced to minimal, therefore, variation among seed sources in field emergence and growth may be interpreted as genetic. Sniezko and Stewart (1989) were of the view that the provenance, and within provenance variation in nursery traits is essentially genetic in nature.

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TRACE METAL ACCUMULATION IN VEGETABLES GROWN IN INDUSTRIAL AND SEMI-URBAN AREAS – A CASE STUDY

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Abstract. The main sources of trace metals to plants are the air or soil media from which trace elements are taken up by the root or foliage. The air, soil and vegetable samples were collected from Industrial, Semi-urban and rural areas and were analysed for Pb, Zn, Ni and Cu. The rural area is free from contaminant sources and is treated as control. From each representative area composite samples of Tomato, Lady's finger, Capsicum and leafy vegetable Bimli were collected and analysed for Pb, Zn, Ni and Cu. The air environments in Industrial and Semi-Urban areas are enriched with the four trace metals, but the concentrations were within the permissible levels. This indicates that, despite the close proximity of the agricultural lands to high emitting industrial sources, soils do not seem to have been contaminated by atmospheric deposition. Remarkable differences were observed between the trace metal content in vegetables of rural areas with semi-urban and industrial areas. In industrial area Nickel, Zinc were reported in higher concentrations in tomato and capsicum where as in semi urban area the concentration of Cu is 2-3 times higher in tomato and lady's finger on comparison with the rural vegetables. Based on the air accumulation factor and concentration factor calculations, the trace metals of Pb and Zn in industrial and semi-urban areas were found to be receiving the contributions from both atmospheric and soil inputs in all the four crops.

Keywords: *trace metals, vegetables, air accumulation factor, concentration factor*

Introduction

Rapid industrialization and urbanization during this century resulted in an increase of metal contamination in soils. The main sources of trace metals to plants are the air or soil from which metals are taken up by the root or foliage. Some trace metals are essential in plant nutrition, but plants growing in a pollute environment can accumulate trace elements at high concentrations, causing a serious risk to human health. (Huchabee *et al.*1983; Hovmand *et al.*1983; Kabata-pendias and Pendias, 1984; Alloway, 1990; Vousta *et al.* 1996; Sharma *et al.*2004).

The uptake of metal concentration by roots depends on speciation of metal and soil characteristics and type of plant species etc. Consequently, metal mobility and plant availability are very important when assessing the effect of soil contamination on plant metal uptake, as well as translocation and toxicity or ultra structural alterations (Luo and Rimmer,1995; Sresty and Madhava Rao,1999; Chandra sekhar *et al.* 2001).

Atmospheric metals are deposited on plant surfaces by rain and dust. Several authors have shown a relationship between atmospheric element deposition and elevated element concentrations in plants and topsoils, especially in cities and in the vicinity of emitting factories (Andersen *et al.*1978; Pilegaard, 1978; Chirgawi, 1989;

Larsen *et al.* 1992; Sanchez *et al.* 1994; Harrison and Vousta *et al.* 1996; Srinivas *et al.* 2002). Airborne submicron particles are also filtered out on plant surfaces, constituting a substantial, but unknown, contribution to the atmospheric supply. Indirect effects of air pollutants through the soil are also great interference, because of the large – scale sustained exposure of soil to both wet and dry depositions of trace elements.

Widespread interest in trace metal accumulation in plant systems has emerged only over the last three decades, and several research articles reported concentrations of a number of trace elements in the local crops and other plants as a consequence of anthropogenic emissions (Bernhard *et al.* 2004^a; Bernhard *et al.* 2004^b; Wong *et al.* 2001). The present study was aimed to find out the trace metal concentration in vegetables grown in industrial and semi-urban areas in relation with air and soil growth media.

Materials and methods

Study Area

Visakhapatnam, often called a city of destiny, has been selected for the case study since numerous sources emit trace metals including several major and minor industries located within the city. Majority of industries such as refineries, fertilizers are located in industrial and semi-urban areas. In both the areas the vegetables are cultivated in a close proximity to the emission sources of trace metals. Basing on the field study, three study areas were selected representing different environmental backdrops. The selected sites are Auto Nagar (Industrial area), Rajeev Nagar (Semi – Urban area) and Chintala Agraharam (Rural or Control area). The samples were collected for a period of two years from 2000 – 2002 (*Fig.1.*). Air and Soil samples were collected bimonthly and vegetable samples were collected seasonally and analyzed for trace metals in air, soil and vegetables.

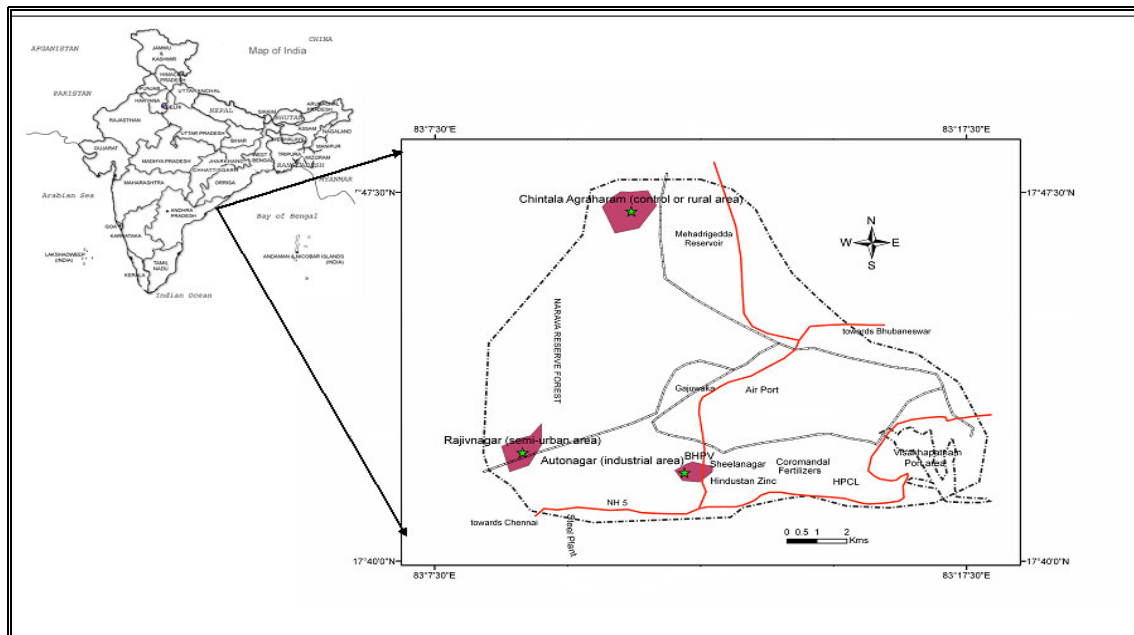


Figure 1. Location map of Visakhapatnam showing study areas

Suspended Particulate Matter (SPM) are collected bimonthly by using High Volume Samplers (HVS) (Envirotech, APM - 420) from two stable residential communities for 8 hours duration (9-00A.M to 5-00 P.M) from each representative area (i.e. Industrial, semi-urban and rural) for two successive years. The collected air particulate matter treated with concentrated HNO₃ acid for elemental analysis (Miroslav and Vladimir 1999). Field experiments were conducted during pre-monsoon (February - May) and post-monsoon (July - January) seasons for two successive years. From each representative area composite samples of vegetables like Tomato (*Lycopersicon esculentum* Mill), Lady's finger (*Abelmoschus esculentus* Linn.), Capsicum (*Capsicum annum* Linn.) and leafy vegetable Bimli (*Hibiscus cannabinus* Linn) are collected from different sampling sites.

For trace metal analysis from each harvest a total of 20 plants were taken at random for analysis. The dried plant samples were digested with HClO₄ and Conc. HNO₃ for trace metal analysis by using Atomic Absorption Spectrophotometer and Inductively Coupled Plasma Emission Spectroscopy (ICP -ES).

The Air Accumulation Factor and Concentration factor were calculated to find out the origin of trace metals in vegetables by using the following formulas (Chandra sekhar *et al.* 2001).

Equations

A. A. F. =

$$\frac{\text{Conc. of metal in Vegetable grown in Indu. Area} / \text{Conc.of metal in Air of Indu. area}}{\text{Conc. of metal in Vegetable grown in Cont. area} / \text{Conc. of metal in Air of Cont. area}}$$

C. F. =

$$\frac{\text{Conc. of metal in Vegetable grown in Indu. Area} / \text{Conc.of metal in Soil of Indu. area}}{\text{Conc. of metal in Vegetable grown in Cont. area} / \text{Conc. of metal in soil of Cont. area}}$$

Statistical Analysis

Statistical significance observed differences between samples was determined by Student's *t*-test and ANOVA test. Differences were considered to be significant at $p \leq 0.05$ and highly significant at $p \leq 0.001$, level of significance.

Results and Discussion

Trace metals in air

The air borne particulate matter in industrial & semi-urban environments found to be highly enriched with trace metals such as Pb, Zn, Ni and Cu as given in *Table 1*.

However, the concentration of the four metals in the air was found to be within the tolerance limits (Mudakavi and Narayana, 1998). The Pb concentration in industrial area reported as 1.46 $\mu\text{g}/\text{m}^3$. whereas the Zn, Ni and Cu concentrations were reported higher in semi-urban area compared with industrial area. The four trace metals of industrial and semi-urban area have shown significant difference (>0.05) with control (rural) area. It may be due to the deposition of air pollutants at higher rates resulting from the dispersion of atmospheric pollutants.

Table 1. Trace metal content in Suspended Particulate Matter ($\mu\text{g}/\text{m}^3$) and soil (mg/kg).

Study area	Air				Soil			
	Pb	Zn	Ni	Cu	Pb	Zn	Ni	Cu
Industrial area Auto Nagar	1.467* ± 0.41	2.453* ± 0.93	0.496** ± 0.15	0.414** ± 0.99	47.8** ± 3.30	49.7** ± 5.24	28.98** ± 2.50	31.1** ± 3.28
Semi Urban area (Rajeev Nagar)	1.376* ± 0.52	4.985** ± 0.87	0.977** ± 0.34	0.648** ± 0.14	15.1** ± 2.4	42.9** ± 6.3	16.8** ± 2.7	11.3** ± 0.91
Rural area C-Agraharam	0.689 ± 0.53	1.691 ± 0.30	0.087 ± 0.01	0.116 ± 0.02	8.1 ± 0.82	32.2 ± 1.75	8.7 ± 0.5	7.7 ± 0.45

* Significant at 0.05 level

** Significant at 0.001 level

n = 12

Trace metals in soils

The trace metal concentrations in soils of industrial, semi-urban and rural areas are given in *Table 1*. The trace metal concentrations of Pb, Zn, Ni and Cu in industrial and semi-urban soils were statistically significant at 0.05 level over control (rural) area and the concentrations are within the permissible limits for agricultural soils (Alloway, 1990; Aswathanarayana, 1999). This indicates that, despite the close proximity of the cultivated land to high – emitting industrial sources, agricultural soil does not seem to have been significantly contaminated by atmospheric deposition. This may be due to low deposition rate resulting from the dispersion of atmospheric pollutants and variations in soil physico – chemical characteristics.

Trace metals in vegetables

The Pb, Zn, Ni and Cu., metal concentrations in the vegetables of Tomato, L. Finger, Capsicum and Bimli were examined during the present study (*Figs. 2, 3*).

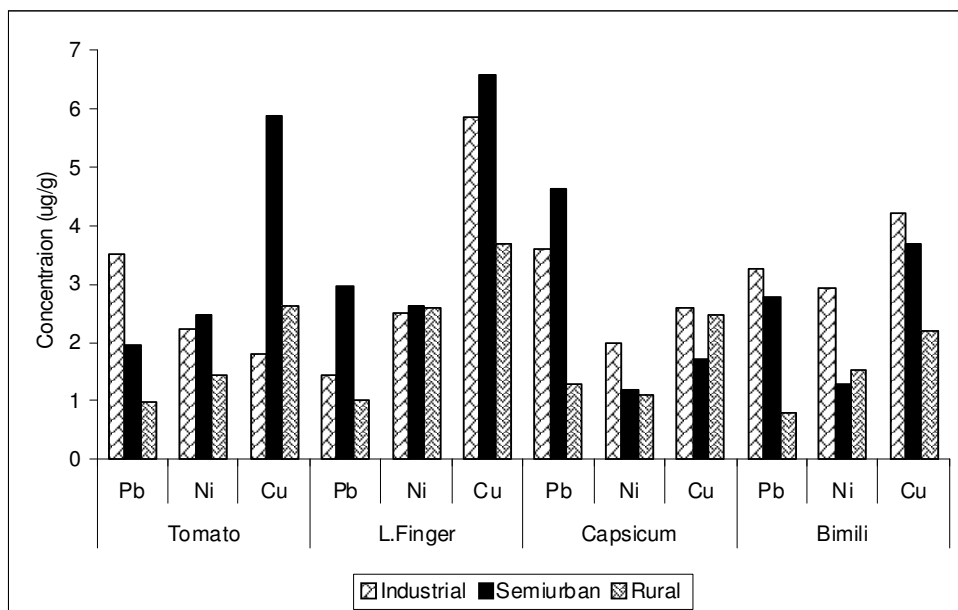


Figure 2. Lead, Nickel and Copper content in Vegetables

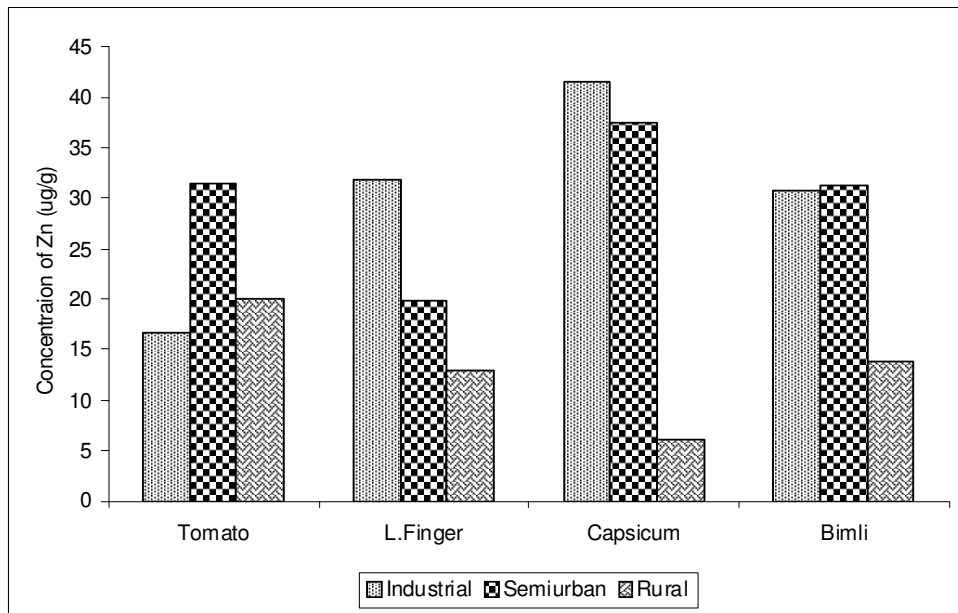


Figure 3. Zinc content in Vegetables

Lead

In general, Pb concentrations in vegetation have increased in recent decades owing to human activities. The Pb content of edible parts of plants growing in uncontaminated areas generally range from 0.05 to 3.0 µg/g d.w. In the present study rural area reported Pb concentration in vegetables in the range of 0.88 to 1.02 µg/g in all the four crops. Whereas in industrial and semi-urban areas the Pb concentration in vegetables in the range of 1.72 to 4.63 µg/g and the average concentration is 3.55 µg/g. It is clearly showing that, the air or soil of urban environments is contributing the Pb by various sources. The permissible limit of lead in vegetables for human consumption is 2.0 - 2.5 µg/g dry weight (Samara *et al.*1992). In the present study the Pb concentration in vegetables cultivated in industrial and semi-urban areas reported above the permissible levels. Hence, it is necessary to kept lead levels as low as possible in the two environments.

Zinc

Environmental pollution of Zn greatly influences the concentrations of this metal in plants. In ecosystems where Zn is an airborne pollutant, the tops of plants are likely to concentrate more Zn on the other hand; plants grown in Zn contaminated soils accumulated a great proportion of the metal in roots (Kabata and Pendias,1992). In the present study the Zn concentration in vegetables grown in industrial and semi – urban areas reported in the range of 19.93 to 41.46 µg/g and in rural area in the range of 12.99 to 20.01 µg/g. Among the four vegetable crops, capsicum and leafy vegetable Bimli accumulated higher levels of Zn. In all the study areas the Zn concentration reported in the four vegetables are within the permissible limits (i.e. 10-50 µg/g) for human consumption (Samara *et al.*1992).

Nickel

Vegetables have more nickel than animal products. The concentration of Ni in plants generally ranges from 0.05 to 5µg/g dry weight. According to WHO (1984) the

Ni concentration in vegetables and fruits reported in the range of 0.02 to 2.7 $\mu\text{g/g}$. The elevated concentrations of Nickel and Copper in plant tissue reflect man made pollution. The Ni content in vegetables from the industrial and semi – urban areas of Visakhapatnam did not show large variability between the study areas. In the present study the Nickel concentration in industrial and semi-urban areas reported in the range of 1.29 to 2.62 $\mu\text{g/g}$. whereas in rural area it was reported in the range of 1.1 to 1.53 $\mu\text{g/g}$. These concentrations of Ni in industrial and semi-urban areas were found at concentrations normally observed in vegetables grown in uncontaminated soils.

Copper

Copper is essential trace element to plants and the amount of copper present in plants varies with the copper content of soil on which it is grown. The copper concentration in food stuffs reported in the range of 1.75 to 9.26 $\mu\text{g/g}$. (Nath *et al.*1982). In the present study the copper content in Semi-urban area reported in the range of 1.7 to 6.58 $\mu\text{g/g}$ with an average of 4.46 $\mu\text{g/g}$. whereas in the industrial and rural areas the average Cu concentration reported as 3.60 and 2.74 $\mu\text{g/g}$ respectively. The concentration of Cu in plants varied much with dependent near by factors like proximity industries and use of fertilizers and Cu based fungicides. The maximum permissible limit for Cu in vegetables is 50 $\mu\text{g/g}$ (Samara *et al.*1992.) and in the present study the concentration of Cu well within the limits in all the four vegetables.

The trace metals Pb, Zn, Ni and Cu have shown variations in concentration among the vegetables and sampling sites. In the present study, the concentration of Zn, Ni and Cu trace elements in vegetables of Tomato, Lady's finger, Capsicum and leafy vegetable Bimli is low and comparable with the results of Samara *et al.* (1992). But remarkable differences were observed between the trace metal content of rural areas with semi-urban and industrial areas. In industrial area Ni, Zn was reported in higher concentrations in tomato and capsicum compared with control areas. In semi-urban area the concentration of Cu in tomato and lady's finger reported 2-3 times higher than the rural vegetables. It was known that the trace metal concentrations in harvested vegetables often show large variation from year to year, even at the same location in the field. This is probably due to variable emission rates, atmospheric transport and deposition process, and plant uptake. (Vousta *et al.*1996). The increased value of trace metal was determined for Pb

in vegetables grown in industrial areas. The four trace metals concentrations in industrial and semi-urban areas have shown a significant difference with control (rural) area.

Air accumulation factor and Concentration factors

The origin of trace metals in vegetables is accumulation due to atmospheric deposition or transfer from soil or both. The AAF and CF values are given in *Table 2*.

In the case of Pb and Zn both AAF and CF factors are high in all the four crops grown in industrial and semi-urban areas. The Ni and Cu have not shown any significant trend and the values are more or less similar in all the four crops. The Pb and Zn are contributing in considerable levels by both soil and air media in all the four vegetable species. However, the variation in AAF and CF values can be ascribed to a number of factors such as chemical speciation of trace metals, atmospheric and soil concentrations and variation in uptake etc. (Vousta *et al.*1996).

Table 2. Air Accumulation Factors (AAF) and Concentration Factors (CF) in vegetable crops at different study areas

Crop	Trace metal	AAF		CF	
		Industrial	Semi-Urban	Industrial	Semi-Urban
Tomato	Pb	1.665	0.981	0.601	1.050
	Zn	0.574	0.533	0.540	0.846
	Ni	0.270	0.151	0.463	0.882
	Cu	0.190	1.081	0.168	4.117
Capsicum	Pb	0.662	1.457	0.246	1.561
	Zn	7.350	2.261	6.911	5.005
	Ni	1.167	0.089	0.287	1.916
	Cu	0.443	0.319	0.391	1.215
L. Finger	Pb	1.297	1.783	0.468	1.911
	Zn	4.693	2.082	4.411	4.607
	Ni	0.314	0.097	0.537	0.564
	Cu	0.293	0.123	3.852	0.470
Bimli	Pb	1.913	1.727	0.690	1.851
	Zn	1.537	0.771	1.445	1.707
	Ni	0.337	0.075	0.576	0.436
	Cu	0.533	0.299	0.471	1.140

Correlation analysis

Correlation analysis applied among sampling sites, vegetable plants and among trace elemental concentrations in vegetables. The data was presented in Table 3.

Table 3. Correlation analysis among study sites, trace metals and vegetables.

n = 12	Industrial	Semi - urban	Rural
Industrial	1		
Semi – urban	0.882**	1	
Rural	0.679*	0.801**	1

n =12	Lead (Pb)	Zinc (Zn)	Nickel (Ni)	Copper (Cu)
Lead (Pb)	1			
Zinc (Zn)	0.619*	1		
Nickel (Ni)	0.038	0.121	1	
Copper (Cu)	-0.142	0.221	0.454	1

n =12	Tomato	Lady's finger	Capsicum	Bimli
Tomato	1			
Lady's finger	0.751**	1		
Capsicum	0.735**	0.931**	1	
Bimli	0.846**	0.950**	0.970**	1

* Significant at 0.05 level, ** Significant at 0.01 level

High significant correlation coefficients were found between the sampling sites of industrial and semi urban, semi-urban and rural. Correlation between industrial and rural region also exist at 0.05 level. This attributes various industrial and urban pollution sources may effect the trace metal composition in vegetables by soil and air pollution.

Among four trace metals, strong correlation was found between Pb and Zn and no correlation with Ni and Cu. This is probably due to variation in uptake mechanism by plants. A strong correlation between all four vegetables species observed. It indicates that the chemical composition of growth media is the major factor influencing the chemical composition of plants.

Conclusion

Among the four trace metals concentrations of Zn, Ni and Cu in vegetables were found relatively low in industrial and semi-urban areas and their concentrations are within the permissible limits for human consumption. The average Pb concentrations in vegetables grown in industrial area reported in higher levels ($>2.5 \mu\text{g/g}$) in all the four crops and it is necessary to kept Pb levels in both air and soil environments as low as possible.

The trace metal content of the agricultural soils was within the standard limits for agricultural soils. However, rising levels of trace metals are observed in industrial and semi-urban soils compared to rural soils. The air borne particulates in industrial and semi-urban areas are enriched with trace metals particularly Pb, Zn, Ni and Cu and are being emitted by various industrial and transport sectors.

The AAF and CF factors for Pb and Zinc are high in industrial and semi-urban areas and contributing those trace elements in to vegetables through air or soil or both. However different vegetable species accumulate different metals, depending on plant available metal species/forms of heavy metals rather than the total concentration in the soil. Dietary intake of leafy vegetables constitutes a major source of accumulation of heavy metals in human body. The detrimental impact of these metals becomes apparent only after decades of exposure.

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EFFECT OF PERIOD OF CULTIVATION ON THE ABUNDANCE AND DISTRIBUTION OF WEED SEEDS IN THE SOIL PROFILE

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Abstract. An experiment was laid down in the greenhouse to determine the distribution of weed seeds at different soil depths and period of cultivation on the distribution of weed seed bank in the soil profile in Muzarabani. Soil samples from different depth levels (0-10, 10.1-20, 20.1-30 cm) were collected soon after harvesting from three different sites namely Gutsa, Mufudzi and Muringazuva of Muzarabani district. The soil samples were put in trays in the greenhouse and watered after every two days to field capacity. Weed seedlings were identified, counted, recorded and discarded for a period of 3 months. Soil samples were disturbed every week to allow more germination of dormant weeds. Depth ($P=0.000$) and site ($P=0.005$) had highly significant effects on the total number of weeds that emerged from the soil samples. The 0-10 cm of the soil had the highest weed seedlings that emerged. There was an equal weed seed distribution in the 10-20 cm and the 20-30 cm of the soil. Gutsa had the largest seed bank and there was no significant difference in seed bank size between Mufudzi and Muringazuva. Some weed species were site specific, *Senna obtusifolia* was only found in Muringazuva and absent in other sites. *Eleusine indica* was found in Mufudzi and was absent in other sites.

Keywords: *weed, soil, greenhouse, germination*

Introduction

Weeds are a menace in agricultural systems the world-over and their management has squandered enormous human and financial capital since the beginning of plant domestication. Extensive research has been done to address this problem and various management packages have been formulated (Clements, Weise and Swanton, 1994). On the contrary, weeds have continued to proliferate and to perfect their survival mechanisms.

Most of the annual weeds ensure their survival through production of large numbers of seed within a short time which can be dispersed through contaminated crop seeds, animal feed, manure and water or transported to distant places on animal hair, fur or wool as well as cultivation and harvesting machinery (Akobundu, 1987). Some of the dispersed weed seeds end up on the soil surface or in the soil. Therefore, the success of weeds is enhanced by the soil seed reservoir that is the major source of weed infestation in most tilled agricultural soils (Altieri and Liebman, 1988). The numbers, types and distribution of weed seeds in their reservoir are determined by the fields location and cropping history, edaphic characteristics such as moisture holding capacity and past

weed control practises (Janiya and Moody, 1989) as well as tillage, land preparation methods and weed seed dormancy (Zimdahl, Moody, Lubigan and Castin, 1988).

Weeds compete for soil mineral nutrients, water and light resulting in 'leggy' cotton, poor plant development, delayed maturity and reduced yields if the weeds are not removed by up to four hoe weedings per season (Cotton Handbook, 1985). Two hoe weedings per season are carried out in each maize field in Muzarabani in order to get a reasonable yield. Late weeds interfere with picking, and weed seeds shed and trash from cotton leaves on cotton lint may result in downgrading (Cotton Handbook, 1985).

Because of the persistence of seeds in the seed-bank, weeds are a major problem in Muzarabani and there have been reports of stagnant or reduced yields despite the use of superior varieties and improved farming methods. Average cotton yields are reported to be around 1 tonne per ha while maize yields are about 0,7 tonnes per ha (Department of Agriculture Technical Extension Services, 1999).

Since one of the major factors affecting annual weed population stability is the large and potentially transient seed-bank, it means seed banks are of ecological and evolutionary importance in the dynamics of weed population and communities. Barralis and Chadoeuf (1987), have found that with the exception of weeds with large seeds, the seed-bank is a better indicator of the long-term influence of agronomic practices on weeds than the above ground vegetation. Knowledge on weed seed distribution in different soil layers makes it possible to develop appropriate approaches and techniques for tillage and long-term weed management. Hence for sustainable cropping, it is important to acquire knowledge on the biological and ecological behaviour of weeds to develop guidelines for environmentally sound weed control (Altieri, 1998; Zimdahl, 1998). In this respect, knowledge of the weed seed-bank is very important because it provides evidence of past field management and may allow forecasts on future weed problems (Wilson *et al.*, 1985 and Forcella, 1992).

Objectives

The objectives of the study were to:

(a) determining the effect of soil depth and period of cultivation on the distribution of weed seedbank in soil profile in the lower Zambezi valley.

Materials and Methods

Three fields with different soil types (alluvial soils, sandy loam, cracking vertisols) and different periods of cultivation (>20 years, >10 years and <5 years) respectively were selected in lower Zambezi valley. A total of 10 randomly selected soil samples were collected from 0 - 10 cm, 10.1 - 20 cm, and 20.1 -30 cm depth of the soil profile in September 2000. The soil cores of the same depth were bulked together and passed through a 2 mm sieve to remove non-reproducing vegetation material and stones. The samples were spread in asbestos trays of 55cm x 30cm which were thoroughly cleaned before soil samples were incubated to make sure that there was no contamination of soil samples with herbicides, since the trays have been used for various purposes before. Soil samples from each sampling position were randomly allocated to the asbestos trays to make a replicate. Soil samples in asbestos trays were watered after every two days to field capacity with distilled water. The weeds that germinated were identified by species, counted and discarded. The total number of weeds that emerged was considered

as transient weed seed bank was expressed m^{-2} . The soil samples were disturbed for three months to allow more germination of weed seeds in the soil. The total number of weeds that emerged was considered as transient weed seed-bank. The experiment was laid out as factorial in a randomised complete block design with three treatments replicated five times. The factors were the depth of soil samples with three levels (0-10 cm, 10.1-20 cm, 20.1-30 cm) and site from which the soil samples were collected with three levels (Mufudzi, Gutsa and Muringazuva). Actual data were transformed using square root transformation before ANOVA to increase homogeneity of error variances. The statistical package used was Minitab version 5.1.

Results

All weeds

Site ($P=0.005$) and depth of soil sample ($P=0.000$) had highly significant effects on the number of all weeds that emerged from incubated soil samples. There was no interaction between site and depth of soil sample ($P>0.05$). Gutsa site had the highest number of weeds emerging from the collected soil samples (Table 1). Muringazuva and Mahwenda site had similar numbers of germinable weeds within their seed banks.

Table 1. Effect of site on the number of germinable weed seeds (square root transformed)

Site	
Gutsa	9.46a (157.07)
Mufudzi	3.80b (21.53)
Muringazuva	5.86b (61.93)

	Significance	S.E.D	L.S.D _{0.05}
Effect of site	P= 0.005	1.61	3.28
Effect of depth	P=0.000	1.61	3.28
Site x depth	NS	NS	NS

Highest number of weeds emerged from the 0-10 cm incubated soil samples (Fig 1.). The 10-20 cm and 20-30 cm depth levels had similar numbers of germinable weeds.

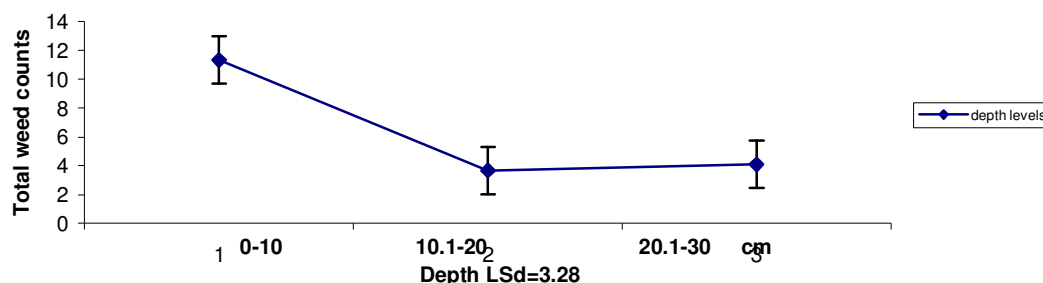


Figure 1. Effect of depth on total weed emergence

Individual weeds

Portulaca oleracea

Site ($P>0.05$) and depth ($P>0.05$) had no effect on the seed bank of *Portulaca oleracea*. There was no interaction between site and depth ($P>0.05$).

Richardia scabra

Site ($P=0.000$) and depth of soil sample ($P=0.008$) had highly significant effects on *Richardia scabra* emergence from incubated soil samples. There was a significant interaction between site and depth of soil sample ($P=0.052$). *Richardia scabra* was more abundant in the soils at Gutsa site than at Mufudzi and Muringazuva. *Richardia scabra* was more abundant at 0-10 cm depth than at 10-20 cm and 20-30 cm depth (Fig 2).

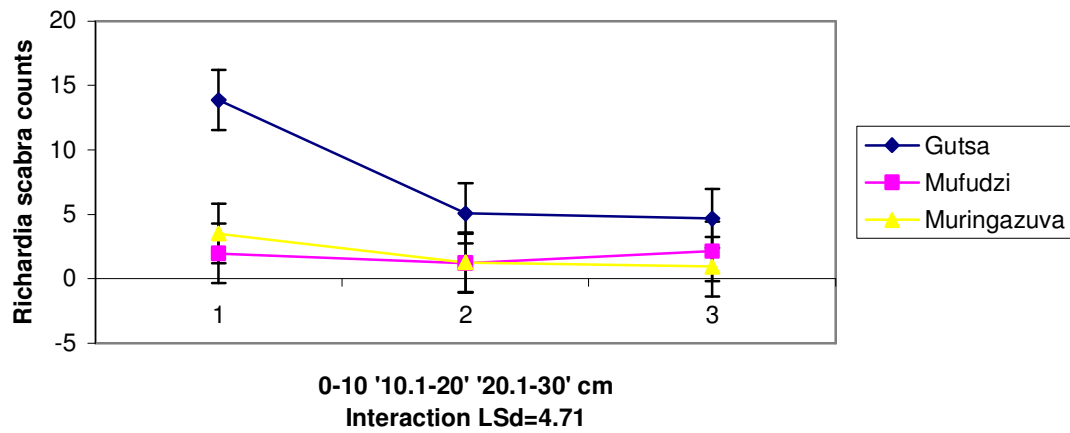


Figure 2. Effects of depth and site on emergence of *Richardia scabra*

The interaction of site and depth on *Richardia scabra* emergence is seen in the different extent to which *Richardia scabra* numbers decreased with increasing depth at the soil samples. For Gutsa site, there was a precipitous decrease in *Richardia scabra* with increase in depth from 0-10 cm to 10-20 cm. The reduction in *Richardia scabra* emergence was not as sharp as Mufudzi and Muringazuva (Fig 2).

Urochloa panicoides

Depth of soil sample ($P=0.000$) had highly significant effects on *Urochloa panicoides* emergence from incubated soil samples. Site ($P>0.05$) had no effect on the seed bank size of *Urochloa panicoides*. There was no interaction between site and depth ($P>0.05$). *Urochloa panicoides* was more abundant in the 0-10 cm than the 10-20 cm and 20-30 cm depth (Fig 3). There was a rapid decline in the numbers of *Urochloa panicoides* as depth was increased from 0-10 cm to 10-20 cm depth. The 10-20 cm and 20-30 cm depth had equal numbers of *Urochloa panicoides* that germinated.

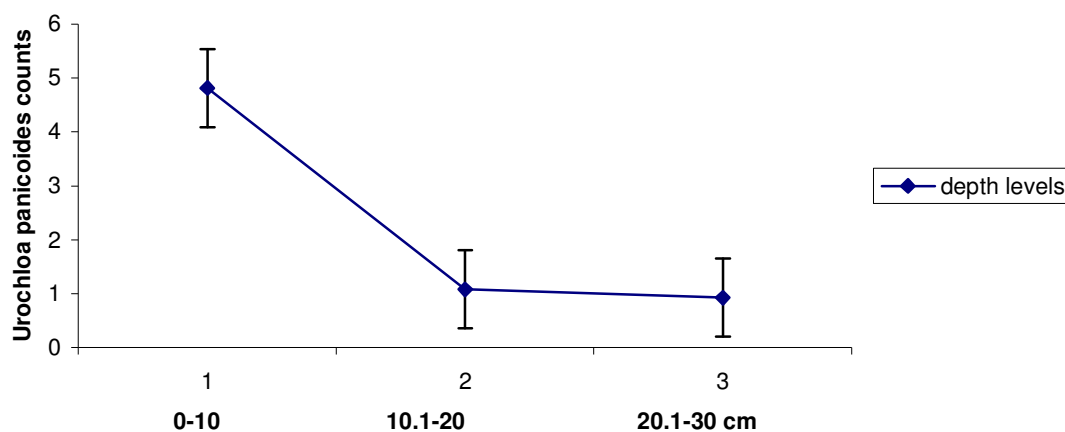


Figure 3. Effect of depth on *Urochloa panicoides* emergence

Nicandra physaloides

Site ($P>0.05$) had no effect on the seed bank size of *Nicandra physaloides*. Depth of soil sample ($P=0.043$) had significant effects on *Nicandra physaloides* emergence. There was no interaction between site and depth of soil samples ($P>0.05$). High numbers of *Nicandra physaloides* emerged from the incubated 0-10 cm of the soil samples. The 10-20 cm and 20-30 cm had equal numbers of *Nicandra physaloides* that emerged (Table 2.).

Table 2. Effect of depth on the number of germinable *Nicandra physaloides*

Depth (cm)	Means		
0-10	1.8510a (5.60)		
10-20	0.8299b (0.40)		
20-30	1.0322b (1.13)		
	Significance	SED	LSD _{0.05}
Effect of site	NS	NS	NS
Effect of depth	P=0.043	0.41	0.84
Site x depth interaction	NS	NS	NS

Corchorus olitorus

Site ($P>0.05$) and depth of soil sample ($P>0.05$) had no effect on the seed bank of *Corchorus olitorus*. There was no interaction between site and depth ($P>0.05$).

Cynodon dactylon

Site ($P=0.006$) and depth of soil sample ($P=0.001$) had highly significant effects on *Cynodon dactylon* emergence from the incubated soil samples. There was a significant interaction between site and depth of soil sample ($P=0.044$). *Cynodon dactylon* was more abundant at the 0-10 cm depth at Gutsa and Mufudzi than the 10-20 cm and 20-30 cm depth of the soil samples. The interaction of site and depth on *Cynodon dactylon* is

seen in the different extent to which *Cynodon dactylon* number decreased with increasing depth of the soil samples. For Gutsa and Muringazuva, there was a sharp decrease in *Cynodon dactylon* numbers with increase in depth from 0-10 cm to 10-20 cm. The decrease in reduction in *Cynodon dactylon* emergence was not very sharp at Mufudzi (Fig 4.)

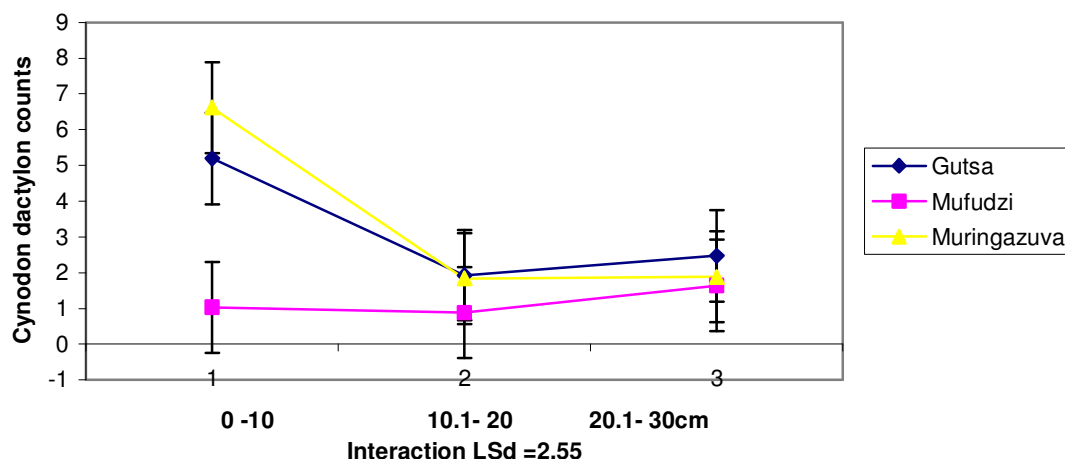


Figure 4. Effect of site and depth on *cynodon dactylon* emergence

Elusine indica

Site ($P>0.05$) and depth of soil sample ($P>0.05$) had no effect on the seed bank of *Elusine indica*

There was no interaction between site and depth ($P>0.005$)

Trichodesma zeylanicum

Depth of soil sample ($P>0.05$) had no significant effect on the emergence of *Trichodesma zeylanicum* from incubated soil samples. Site ($P=0.02$) had significant effects on the seed bank of *Trichodesma zeylanicum*. Muringazuva had the highest number of *Trichodesma zeylanicum* that emerged. Gutsa and Muringazuva had equal numbers of germinable *Trichodesma zeylanicum* (Table 3.). There was no interaction between site and depth of soil sample ($P>0.05$).

Table 3. Effect of site on the number of germinable *Trichodesma zeylanicum*

Site	Means		
Gutsa	0.7071b (0.00)		
Mufudzi	0.9436b (0.60)		
Muringazuva	1.1777a (1.33)		
	Significance	SED	LSD _{0.05}
Effect of site	P=0.02	0.16	0.32
Effect of depth	NS	NS	NS
Site x depth interaction	NS	NS	NS

Senna species

Site ($P>0.05$) and depth of soil sample ($P>0.05$) had no effect on the seed bank of *Senna* species. There was no interaction between site and depth of soil sample ($P>0.05$) (Appendix 1. 10).

Overall weedbank in all three sites

The overall weed seed bank in the three sites was made up of nine weed species, with a prevalence of broadleaved weeds. Ranking of weeds was done (Table 4), weed species with the highest mean ranked as one and weed species with lowest mean ranked as nine. *Richardia scabra* was the dominant weed species in Gutsa village. *Portulaca oleracea* was dominant in Mufudzi and *Cynodon dactylon* was dominant in Muringazuva. Some weed species were site specific. *Senna* species was found in Muringazuva only. *Eleusine indica* was found in Mufudzi village only. *Trichodesma zeylanicum* was also found in Mufudzi area and Muringazuva and was absent in Gutsa village.

Table 4. Summary of abundance and distribution of weed species in the three sites (transformed data)

SPECIES	Gutsa	Rank	Mufudzi	Rank	Muringazuva	Rank	Sign.
<i>Portulaca oleracea</i>	2.51	3	1.82	1	1.09	6	P=0.169
<i>Ricardia scabra</i>	7.88	1	1.76	3	1.91	3	P=0.000
<i>Urochloa panicoides</i>	2.13	4	1.79	2	2.90	2	P=0.41
<i>Nicandra physaloides</i>	1.13	5	0.78	7	1.66	4	P=0.203
<i>Cynodon dactylon</i>	3.2	2	1.18	4	3.45	1	P=0.006
<i>Eleusine indica</i>	0.71	7	0.88	8	0.71	9	P= 0.248
<i>Trichodesma zeylanicum</i>	0.71	7	0.94	5	1.18	5	P=0.02
<i>Senna species</i>	0.71	7	0.71	9	0.93	8	P=0.173
<i>Corchorus olitorus</i>	0.94	6	0.78	7	1.00	7	P=0.437

Value 0.71 means the weed was absent in the original data.

Discussion

In all farmers' fields, about 59.3% of the total weed seed bank was concentrated in the upper 0-10 cm of the soil. Such a large surface seed stock is attributed to the considerable seed rain that occurred during the recent crop cycle because of inadequate weed control. Farmers have a tendency of not weeding late weeds. These late weeds are responsible for enriching the seedbank. Cardina *et al.*, 1992 found that with control of these late weeds and good weed control management throughout the season, the soil seed bank can actually be depleted to result in reduced weed problems in the subsequent years with consequent savings of time and weed control costs. About 19.3% of the total weed seed bank was concentrated in the 10-20 cm and the other 21.4% was concentrated in the 20-30 cm of the soil. Cardina *et al.*, 1992 also found that the top 0-5 cm of the soil had the highest numbers of the total weed seeds. An equal distribution of weed seeds in the 10- 20 cm and 20-30 cm support work done earlier on by Yenish *et al* (1992). Weed seed depth distribution besides being affected by tillage is also affected by soil type with those soil likely to crack (silty clay loam) causing freshly produced seed to be deposited deeper than the non-cracking soils (Cardina *et al.*, 1992). Also of

importance some weed seeds, which are buried deeply by the plough rot or are preyed by micro-organisms hence viability is lost, or have acquired dormancy which required a long period of time to germinate thus fewer seed numbers in the 10-20cm, and 20-30cm depth levels.

Seed bank size was highest in a field with a period of cultivation greater than 20 years. Weeds among other characteristics are known to proliferate quickly in soils that have been disturbed for a long time and are continuously being disturbed. For example *Richardia scabra* is known to favour exhausted soils with a low pH. The differences in the size of the seed-bank among the three farmer's fields can also attributed to by differences in fields location, cropping history, edaphic characteristics such as moisture holding capacity and past weed control practices and tillage, land preparation methods.

Conclusions

The 0-10 cm depth level contains higher weed seeds than the 10.1-20 and 20.1-30 cm depth levels. Fields, which have been cultivated for a long period of time, tend to have a larger seed bank than fields, which have been opened recently.

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USING RESIDUAL ANALYSIS TO VALIDATE RICE SOWING DATES EXPERIMENT MODEL

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Abstract. Picking a model for a problem is a major undertaking. If the model fits well then it can be used to increase understanding of the problem and/or for prediction. Several statistical procedures have been used by researchers for planting date studies to suit their objectives. The ever-proliferation of Statistical procedures available to researchers has given room for use of diverse statistical design for research into finding optimum planting time for crops. Considering the subtle differences, advantages and disadvantage that these statistical designs pose, the results of such analysis may lead to false conclusion or be less reliable at least for comparative purposes. There is need to look at date of planting trials again to see the possibility of proffering a statistical model that could be commonly used by researchers. The main purpose of this work is to apply the residual analysis to check the suitability of the series of similar experimental model to describe the effects of sowing dates on yield of upland rice with the view of predicting optimum sowing date. Results show that the series of similar experiment methodology is able to model the changes associated with different sowing dates. The questions associated with model adequacy were discussed.

Keywords: *Sowing dates, rice, statistical model, residual analysis*

Introduction

Studies on dates of planting have been extensively conducted for several crops across different ecological zones. A review of studies on rice planting dates indicates that these research were necessitated by weather pattern [6, 38]; pest management [5, 18, 32, 36, 37]; disease management [2, 20]; and grain quality [9].

Several statistical procedures have been used by researchers for planting date studies to suit their objectives. Obi [23] proposes series of similar experiment models for time of planting experiments. Some researchers [3, 25, 27], used this series of similar experiment model in their study. A survey of other works on planting date show that [35] used a randomized block design with four complete blocks to study the effects of planting dates and residue rate effect on growth partitioning and yield of corn. Also [2, 18] used randomised complete block design in their planting date trials, while [28] in their trial on response of Soybean lines with “juvenile” trait to day length and sowing date combined both greenhouse and field work. In both cases they used factorial arrangement. Acikgoz [1] in his studies on effect of sowing time and planting method on rice yield per day also used factorial experiment. Fakorede [12], in maize planting date trial, used a randomised complete block design (RCBD) with a split-plot arrangement. He assigned dates to main plot, plant density to sub plot and genotypes to

the sub-sub plot. Chand and Singh [5], used split-plot design in their study of effect of planting time on Stem Rot (SR) incidence in rice, [8] also used split-plot design for his studies. Both [5] and [8] assigned dates to main plot and variety to sub-plot.

Federer Design: Walter T. Federer, Professor of Biological Statistics, Biometrics Unit, College of Agriculture and Life Sciences, Cornell University, Ithaca, New York (2001) (Personal communication with Obi, I. U.) gave his premise for Time (Date) of Planting which is Stated as follows: “Variety x Location, Variety x Year, and Variety x Location x Year interactions are mostly Date of Planting x Variety interaction”. Federer said, “If I were setting up an experiment on Date of Planting, I would use a number of Locations (Different), and Years, and in each Year and Location, I would have a replicated experiment where the replicates were Dates of Planting, say three (3) Dates with two (2) Replicates, each, Whole-plots would be Crops and the Split-plots would be Varieties within Crops”. Some form of an Analysis of Variance (ANOVA) as suggested by Federer is as presented in *Table 1*.

Table 1. Form of partial analysis of variance, showing source of variation only (Walter T. Federer, April 2001, Personal communication in writing).

Source of Variation
“Location = L
Year = Y
L x Y
Dates of Planting = D
Reps within D within L and within Y
D x L
D x Y
D x L x Y
Crops = C
C x L
C x Y
C x L x Y
C x D
C x D x L
C x D x Y
C x D x Y x D
Varieties within Crops, etc.

Verifying the adequacy of the linear model

The outlier tests [10, 15, 30, 34] as well as the variance homogeneity tests [4, 16, 29] have been used. Kirton, personal communication with Federer (1977), Department of Agriculture, New South Wales, Sydney, Australia, as cited by [23], suggested that after one has a “correct model,” one should proceed as follows in searching for a discrepant “treatment” or “block”: (i) *compute estimated residuals*, (ii) *use absolute values of the residuals*, and (iii) *perform a standard analysis of variance (ANOVA)*, the same one as used for the response, and/or multiple comparisons on the *absolute* values of the estimated residuals. If the model is “correct”, then the null hypothesis should be true for all categories in the ANOVA except for residuals of absolute values of residuals. That is, the expected value for each F- test is “one”. If the null hypothesis (hypotheses) is (are) not true, then this procedure can be used to pinpoint discrepant treatments, blocks, etc., in the experiment [13].

Federer *et al.*, [14] did “Studies of Residual Analysis” titled; “Analysis of Absolute Values of Residuals to Test Distributional Assumptions of Linear Models for Balanced Designs”. Although statistical models are largely used in Sowing dates data analysis, a verification of the suitability of the model is not always checked. Hence indicating that the search for the *Model* for the time (date) of planting experiment is continuing and the *Model* and *Field Layout* are yet to be confirmed.

The main purpose of this work is to apply the residual analysis to check the suitability of the conventional series of similar experiment proposed by [23] to describe the effect of sowing dates on rice cultivars. Additionally, the authors expect that this paper is capable of introducing a step-by-step procedure for the implementation of the residual analysis to any statistically significant model. Also answers would be proffered to questions concerning model validity such as:-

- (i) How can I tell if a model fits my data?
- (ii) How can I assess the sufficiency of the functional part of the model?
- (iii) How can I test whether or not the random errors are distributed normally?

Statistical model

A statistical model is a mathematical model which contains a random error with a specific probability distribution. Usually, this model is used to predict the value of one of the variables when the other is known, under specific conditions [31]. In a statistical model, two or more variables are related using regression analysis equations. These equations are mainly used to predict the dependent variable, Y , as a function of the independent variable, X . In the analysis, some assumptions are necessary [7, 11].

The independent variable, X , is considered free of errors because X is not a random variable. There is a linear relationship between Y and X and the statistical model that relates Y_i to X_i is given by:

$$Y_i = A + B X_i + \varepsilon_i \quad (\text{Eq.1})$$

for $i=1, \dots, n$, where n is the number of observations.

In Equation 1, A and B are unknown constants to be estimated and they are called parameters of the regression model. The random value, ε_i is the denominated random error. The value of ε_i for any observation will depend on both a possible error of measurement and other variables different from X_i that were not measured that could affect Y_i . The values of ε_i are random variables, assuming the following assumptions:

- (i) The average of ε_i values is equal to zero and its variance, σ^2 , is unknown and constant for $1 \leq i \leq n$;
- (ii) ε_i values are not correlated;
- (iii) The distribution of ε_i values is normal for $1 \leq i \leq n$.

Second and third assumptions imply that ε_i values are mutually independent.

The regression line is, in general, unknown and therefore must be estimated through the sampling data. In the particular case where the regression of Y in relation to X is linear, the best fit line can be written as:

$$\hat{Y}_i = \hat{A} + \hat{B}X_i \quad (\text{Eq.2})$$

where the symbol "caret" (^) denotes estimate (estimator) \hat{A} and \hat{B} are determined by the least squares method and \hat{Y}_i are the estimated values of Y_i using Equation 2 such that the differences between Y_i and \hat{Y}_i shall be minimum. Generally, these differences are

known as residuals, i.e., errors associated to the predicted values of Y_i corresponding to each X_i value and which can be calculated through the following expression:

$$\hat{e}_i = Y_i - \hat{Y}_i \quad (\text{Eq.3})$$

A discussion of a simple linear model considering two variables X and Y would enhance our understanding of procedures used to examine the adequacy of a model

$$Y = a + bX + e$$

The variable e denotes random error, that is, if there were no error Y would be a deterministic linear function of X .

When is a model good? At first, one might say when there is no error. But for all the data that we consider in this class there will always be error. Actually we will say a model is good if there is no connection between e and $a + bX$; that is, the random error is free of X . Hence, for predicting Y , we have found the model that contains all the information based on X . Now there may be other variables which help in predicting Y . These will be contained in e .

Model Assumption: So the assumption we want to verify on a model is: the random error component is independent of the X component. How would we check this assumption? If we knew the random errors, e , we could just plot them against $a + bX$. A random scatter would indicate that the errors do not depend on $a + bX$; i.e., the errors are free of $a + bX$. Thus the model is good. However, we don't know the errors, we only know Y and X . But using Y and X we estimate a and b . This leads to an estimate of $a + bX$, the predicted value of Y , which we label as \hat{Y} . Our estimate of the error is $Y - \hat{Y}$. This is called the residual, literally, what's left. We will denote the residual by \hat{e} , that is $\hat{e} = Y - (\hat{a} + \hat{b}X)$. Then we can check our model assumption by plotting \hat{e} versus \hat{Y} . This is called the residual plot. A random scatter indicates a good model. If it is not a random scatter then we need to rethink the model [26]. The verification of residuals normality can also be analysed by plots, such as normal score and normal probability graphs. In these graphs, the assumption of normality is valid if the points in the graph are localized approximately along a straight line. However, in case of doubt, the linearity can be confirmed using a statistical test of normality, such as the one proposed by [31].

Experimental Procedures

Field experiments were conducted during the 2002 and 2003 cropping season at three (3) locations within Benue State viz Makurdi ($7^{\circ} 41' 00''N$; $8^{\circ} 37' 00''E$; 100 m above sea level), Otobi near Otukpo ($7^{\circ} 13' 00''N$; $8^{\circ} 9' 00''E$; 105 m above sea level) and Yandev near Gboko ($7^{\circ} 18' 00''N$; $8^{\circ} 41' 00''E$; 180 m above sea level). The plots were sown on June 15 and June 30 (early planting dates) and July 15 and July 30 (late planting dates).

Five (5) lines of rainfed upland rice comprising four newly developed New Rice for Africa (NERICA) lines (WAB 450-11-1-P-31-1-HB, WAB 450-1-B-P-38-I-HB, WAB 450-1-B-P-105-HB, WAB 450-1-B-P-160-HB) and a recommended variety ITA 150 (Standard check) obtained from the West Africa Rice Development Association (WARDA), IITA Ibadan were used for the experiment.

The treatments consist of five rice lines laid out in a Randomised Complete Block designs (RCBD) of three blocks/replications. Each treatment was assigned to an experimental unit of 12m² size (3m x 4m) but data were taken from the inner 6m² (2m x 3m) with 1m left as perimeter border. Replicates and experimental plots were separated length-wise by 1m walk-way and adjacent experimental plots were separated by 0.5m. This arrangement was repeated for each of the four planting dates at the three locations of study. Experimental layout of this form was described as series of similar experiment model (Obi, 2006). The form of analysis for each date of planting showing sources of variation and degrees of freedom (General) and (specific) are shown in *Table 2*. *Figure 1*. showed the field layout consisting of five rice lines, four date of planting as repeated in each of the three locations.

The experimental areas were ploughed and harrowed twice. The seeds were drilled at the rate of 50kg/ha (60g/plot) at 20cm apart; this gave a total of 16 rows of 4m length per plot. Fertilizer was applied at the rate of 75kg N, 60kg P, 60kg K per hectare at three split doses. Basal fertilizer application was done using NPK 15:15:15 brand of compound fertilizer at 30kg N/ha just before the second harrowing. The first top dressing was carried out at five (5) weeks after planting (5 WAP) at the same rate as basal application and using the same brand of fertilizer while the second top dressing was carried out with urea at 15kg N/ha (12 WAP) which corresponded with panicle initiation stage. All the three split fertilizers doses were applied using broadcasted method.

1. Date of Planting d₁

BLOCK I	v ₁	v ₂	v ₄	v ₃
BLOCK II	v ₃	v ₄	v ₂	v ₁
BLOCK III	v ₂	v ₃	v ₄	v ₁

2. Date of Planting d₂

BLOCK I	v ₄	v ₂	v ₁	v ₃
BLOCK II	v ₁	v ₄	v ₃	v ₂
BLOCK III	v ₄	v ₃	v ₁	v ₂

3. Date of Planting d₃

BLOCK I	v ₂	v ₄	v ₃	v ₁
BLOCK II	v ₁	v ₂	v ₄	v ₃
BLOCK III	v ₄	v ₃	v ₂	v ₁

4. Time of Planting d₄

BLOCK I	v ₂	v ₁	v ₃	v ₄
BLOCK II	v ₄	v ₂	v ₁	v ₃
BLOCK III	v ₃	v ₃	v ₄	v ₂

Figure 1. The field layout for Date (Time) of Planting Experiment as Series of Similar Experiments (d₀ = First Date of Planting and d₄ = Date of Planting 8 weeks after)

Table 2. Form of Analysis of Variance for each Date of Planting Showing: Sources of Variations and Degrees of Freedom (General) and (Specific), Only

Source of Variation	d.f. (General)	d.f. (Specific)
Block/Replication	r - 1	2
Varieties	v - 1	4
Experimental Error = Block x Variety	(r - 1)(v - 1)	8
Total	Vr - 1	14

A combination of two post-emergence herbicides (propanil and 2,4-D.) were applied at 4 WAP at a mixture ratio of 2:1 with propanil at 1.6L/ha and 2,4-D at 1.1L/ha using CP 3 knapsack sprayer. Supplemental weed control was manually done using the local hoe and hand picking as was necessary during the season.

Data on yield was collected at maturity; the plants from the inner 2m x 3m (6m²) of each experimental unit (i.e. with 1m left as perimeter border) were harvested, threshed carefully, winnowed and the grains weighed and recorded in kilograms. Grain yield per plot was converted to tonnes per hectare.

The Analysis of Variance was performed using the procedure outlined by [33] for each measured parameter. Means that had a significant F-test were separated using LSD 0.05 [24]. The data analysis was carried out in stages.

Test of Homogeneity of variance

Bartlett's [4] test for homogeneity of variance was conducted in order to determine whether or not the assumption of homogeneity of variance was met [23].

Stage I: The data were separately analyzed for yield for each planting date within a location, where variances were homogenous (i.e. where the experiments showed no significant Bartlett test). This permits the combined analysis of variance for each date of planting.

Stage II: A combined analysis of variance was done for yield measured over date of planting in each location following the procedure of analysis of combined experiments as outlined by [19]. The form of combined analysis for each trait measured for date of planting showing sources of variation and degrees of freedom (General) and (specific) are shown in *Table 3*. The linear statistical model used for the analysis of variance is as

$$Y_{ijkl} = \mu + \rho_j + \alpha_i + \beta_k + (\alpha\beta)_{ik} + \varepsilon_{ijkl}$$

Y_{ijkl} = Observation made on the i^{th} variety within the j^{th} replication in the k^{th} planting date within the l^{th} location

μ = overall experimental mean

ρ_j = effect of the j^{th} Block/replication within time

α_i = effect of the i^{th} variety

β_k = effect of the k^{th} date of planting

$(\alpha\beta)_{ik}$ = Interaction effect of i^{th} variety with the k^{th} planting date

ε_{ijkl} = random unit of i^{th} variation within the j^{th} block within the k^{th} planting date on the i^{th} variety component of experimental error.

i = 1,2, 3, 4 and 5 rice lines.

j = 1,2 and 3 block.

k = 1,2,3 and 4 planting dates.

l = 1,2 and 3 locations

Table 3. Form of Combined Analysis of Variance for Four Date of Planting Showing Sources of Variance, Degrees of Freedom (General) and (Specific) only

Source of Variation	d.f. (General)	d.f. (Specific)
Varieties (V)	$v - 1$	(4)
Date of Planting (D)	$d - 1$	(3)
V x D	$(v - 1)(d - 1)$	(12)
Block within Date	$(r-1)(d)$	(8)
Experimental Error = Block x DV	$d(r - 1)(v - 1)$	32
Total	$rdv - 1$	59

Stage III: A combined analysis of variance was done for yield measured over the three locations. Date of plantings as well as locations were considered to have random effects while varieties were considered as fixed effects. The form of analysis of variance, showing sources of variation and degrees of freedom is presented in *Table 4*.

The linear statistical model used for the analysis of variance is as follows:-

$$Y_{ijkl} = \mu + \alpha_i + \rho_j + \beta_k + \gamma_l + (\alpha\beta)_{ik} + (\alpha\gamma)_{il} + (\beta\gamma)_{kl} + (\alpha\beta\gamma)_{ikl} + \varepsilon_{ijkl}$$

Y_{ijkl} = Observation made on the i^{th} variety within the j^{th} replication in the k^{th} planting date within the l^{th} location

μ = overall experimental mean

α_i = effect of the i^{th} variety

ρ_j = effect of the j^{th} block/replication

β_k = effect of the k^{th} planting date

γ_l = effect of the l^{th} location

$(\alpha\beta)_{ik}$ = Interaction effect of i^{th} variety with the k^{th} planting date

$(\alpha\gamma)_{il}$ = Interaction effect of the i^{th} variety with l^{th} location

$(\beta\gamma)_{kl}$ = Interaction effect of the k^{th} planting date with the l^{th} location

$(\alpha\beta\gamma)_{ikl}$ = Interaction effect of the i^{th} variety on the k^{th} planting date within l^{th} location.

ε_{ijkl} = random unit of variation within the j^{th} replication within the k^{th} planting date within the l^{th} location on the i^{th} variety component of experimental error.

$i = 1, 2, 3, 4$ and 5 rice lines.

$j = 1, 2$ and 3 blocks/replications.

$k = 1, 2, 3$ and 4 planting dates.

$l = 1, 2$ and 3 for locations.

Table 4. Form of combined analysis of variance for three locations and four dates of planting showing Sources of variation (Source), Degrees of freedom (d.f)

Source	d.f. (General)	d.f. (Specific)
Location (L)	(l-1)	2
Dates (D)	(d-1)	3
L x D	(l-1)(d-1)	6
Block/Location	l(r-1)	6
Rice Lines (V)	(v-1)	4
L x V	(l-1)(v-1)	8
D x V	(d-1)(v-1)	12
L x D x V	l(dv-1)(r-1)	24
Residual	lv(r-1)	114

where, l, d, v and r are number of locations, dates of planting, rice lines, and number of blocks, respectively

Results

Series of similar experiment model involve analyses of variance in stages. The example of the results of mean square of the stage I of the analysis of variance and degree of freedom for yield on June 15 is presented in *Table 5*. The example of the results of variance for traits measured from the four dates of planting at Makurdi and the results of Bartlett's test of homogeneity of variance is shown in *Table 6*. The Bartlett's test was not significant. Based on non significant Bartlett's test of homogeneity of variance, the procedure of analysis of combined experiments as outlined by McIntosh (1983) was used to combine the four planting dates at each location. This is the stage II

of the analysis of series of similar experiment model. An example of the results of the combined analyses of the date of planting for each of the location is presented in *Table 7*.

Table 5. Sources of Variation, Degrees of freedom (D.F) and Mean Squares from analyses of variance for yield of five rice lines grown at June 15 at Makurdi, Otobi and Yandev during 2002 and 2003 cropping seasons

Source of Variation	d.f	Mean squares					
		2002			2003		
		Makurdi	Otobi	Yandev	Makurdi	Otobi	Yandev
Block/Replication	2	1.03	3.39	0.40	1.39	3.38	0.43
Varieties	4	0.43	0.49	0.51	0.27	0.45	0.54
Experimental Error	8	0.34	0.73	0.27	0.37	0.71	0.29
Total	14						

*, ** = significant at 5% and 1% respectively.

Table 6. Error Squares (Variances) for Yield of Five Varieties of Rice Planted on fourth-nightly interval (15th June – 30th July, 2002 and 2003) in Makurdi, Otobi and Yandev

Date of Planting	d.f.	Mean squares					
		2002			2003		
		Makurdi	Otobi	Yandev	Makurdi	Otobi	Yandev
June 18	8	0.3369	0.7278	0.2743	0.7264	0.2569	0.2966
July 2	8	1.023	0.4921	0.2390	0.4688	0.2007	0.06184
July 16	8	1.023	0.1208	1.2220	0.09375	0.0938	0.6448
July 30	8	0.8663	1.562	0.5014	0.3070	0.4492	0.7734
X ² Calculated		6.03NS	11.05NS	6.96NS	7.435NS	4.611NS	11.33NS

Critical values of χ^2 at 5%, 8 d.f. = 15.51; Critical values of χ^2 at 1%, 8 d.f. = 20.09
 NS = Not significant.

The Results of the stage III analysis which is the combined analysis of variance for yield of five varieties of rice planted over four sowing/planting dates at Makurdi, Otobi and Yandev locations in 2002 and 2003 are presented in *Table 8*. and their Bartlett's test presented in *Table 9*., respectively. The Bartlett's test was not significant. The results of combined analysis of variance done for each character measured for the two years of study across the four date of planting among the three locations was pooled and is presented in *Tables 10*. The model for this last stage of combined analysis was validated.

Table 7. Combined analysis of variances showing sources of variation, degrees of freedom (d.f) and mean squares from analyses of variance for yield of five varieties of rice planted on fourth-nightly interval (15th June – 30th July, 2002 and 2003) at Makurdi, Otobi and Yandev

Date of Planting	d.f.	Mean Squares					
		Locations					
		2002			2003		
		Makurdi	Otobi	Yandev	Makurdi	Otobi	Yandev
Varieties (V)	4	1.68*	2.49*	3.42**	1.411*	0.66	0.71*
Date of Planting (D)	3	2.92**			7.54**	7.39**	0.15
			13.52**	11.36**			
V x D	12	0.86NS	0.65NS	1.92**	0.76	0.23	0.19
Block within Date	8	2.31**	3.32**	3.09**	1.71**	1.67**	0.46
Error	32	0.61	0.73	0.56	0.44	0.42	0.25
Total	59						

*, ** = significant at 5% and 1% respectively.
NS = non significant.

Table 8. Combined analysis of variance for yield of five varieties of rice planted over four sowing/planting dates at Makurdi, Otobi and Yandev locations in 2002 and 2003

Date of Planting	d.f.	Mean Squares	
		2002	2003
Location (L)	2	0.84**	19.35**
Dates (D)	3	0.16**	8.56**
L x D	6	0.42**	3.26**
Block/Location	6	0.06	1.11
Rice Lines (V)	4	0.24**	2.23**
L x V	8	0.03	0.28
D x V	12	0.05	0.67
L x D x V	24	0.03	0.26
Residual	114	0.04	0.52
Total	179	0.07	0.98

*, ** = significant at 5% and 1% respectively. NS = non significant.

Table 9. Error squares (variances) for yield five varieties of rice planted on fourth-nightly interval (15th June – 30th July,) in Makurdi, Otobi and Yande in 2002 and 2003

Date of Planting	Error d.f.	Error Mean Squares (variances)	
		2002	2003
Otobi	32	1.227	0.42
Yandev	32	1.058	0.25
Makurdi	32	0.9026	0.44
X ² Calculated		0.4596	2.17

Critical values of χ^2 at 5%, 32 d.f. = 43.77

Critical values of χ^2 at 1%, 32 d.f. = 50.89

The R² for this model accounted for 61.3%. The graphical residual analysis of this trial which is given by the graph of residuals plotted against predicted values is presented in *Figure 1*. The plot did not revealed anything particularly troublesome pattern other than a random pattern, although the largest positive residual value observed slightly above 3 stands out from the others.

The normal probability plot is presented in *Figure 2*. The normal probability plots for this indicate that that it is reasonable to assume that the random errors for these processes are drawn from approximately normal distributions.

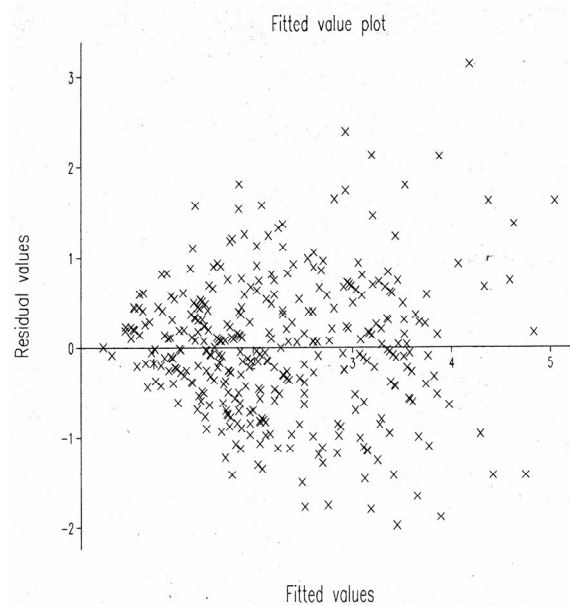


Figure 2. Plot of Predicted Yield (t/ha) versus Yield (t/ha) residual

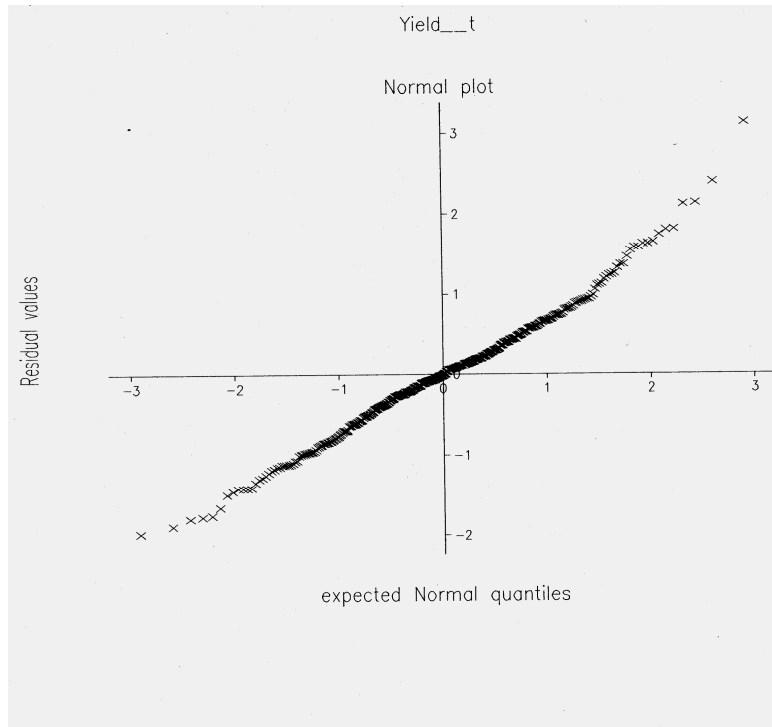


Figure 3. Plot of residual against normal scores for Yield (t/ha)

The Histogram for this study is shown in Figure 4. The histogram is more-or-less bell-shaped.

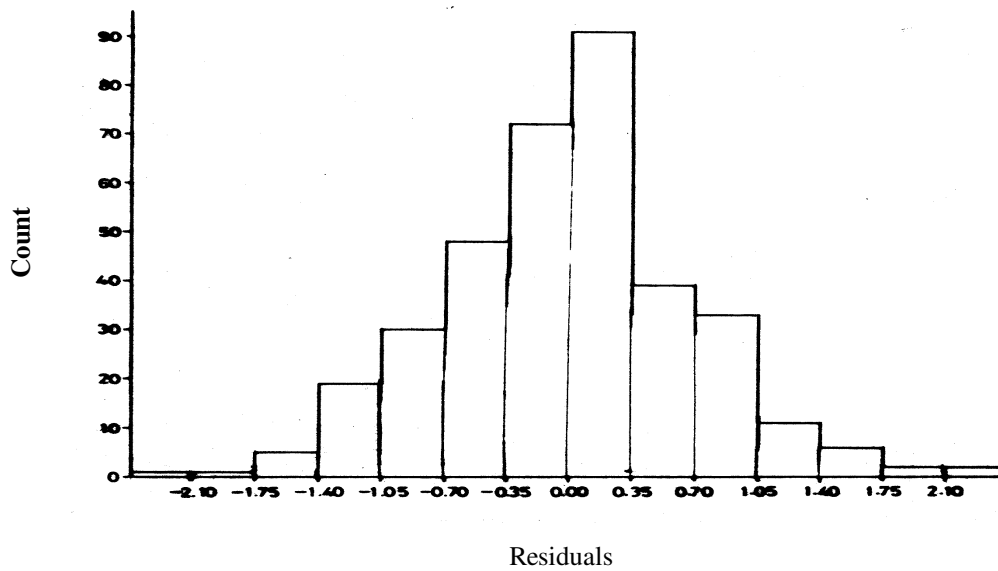


Figure 4. Histogram of residual for yield (t/ha)

Discussion

Model validation is possibly the most important step in the model building sequence. It is also one of the most overlooked. According to Montgomery (1991), before the conclusions from the analysis of variance of a design are adopted, the adequacy of the model should be checked. Often the validation of a model seems to consist of nothing more than quoting the R^2 statistic from the fit (which measures the fraction of the total variability in the response that is accounted for by the model). Unfortunately, a high R^2 value does not guarantee that the model fits the data well. Use of a model that does not fit the data well cannot provide good answers to the underlying scientific question of how one can know if a model fits the data under investigation. Even though the R^2 for this model accounted for 61.3%, the primary statistical tool for most process modeling applications is graphical residual analysis [21]. In addition, the normal probability plot also serves as to confirm the adequacy of a model. [21, 26].

Different types of plots of the residuals from a fitted model provide information on the adequacy of different aspects of the model. Numerical methods for model validation, such as the R^2 statistic, are also useful, but usually to a lesser degree than graphical methods. Graphical methods have an advantage over numerical methods for model validation because they readily illustrate a broad range of complex aspects of the relationship between the model and the data. Numerical methods for model validation tend to be narrowly focused on a particular aspect of the relationship between the model and the data and often try to compress that information into a single descriptive number or test result. If the model's fit to the data were correct, the residuals would approximate the random errors that make the relationship between the explanatory variables and the response variable a statistical relationship. Therefore, if the residuals appear to behave randomly, it suggests that the model fits the data well. On the other hand, if non-random structure is evident in the residuals, it is a clear sign that the model fits the data poorly. The plot did not reveal anything particularly troublesome pattern other than a random pattern, although the largest positive residual value observed slightly above 3 stands out from the others. It is not enough in the scattered plot to indicate unsuitability of the model for the study. According to [21], it is possible that a particular treatment combination produces slightly more erratic response than the others. The problem more over is not severe enough to have a dramatic impact on the analysis and conclusions [21].

The assessment of the sufficiency of the functional part of a model also depends on the scatter plot of the residuals versus the predictor variables in the model and versus potential predictors that are not included in the model. These are the primary plots used to assess sufficiency of the functional part of the model. Plots in which the residuals do not exhibit any systematic structure indicate that the model fits the data well. Plots of the residuals versus other predictor variables, or potential predictors, which exhibit systematic structure, indicate that the form of the function can be improved in some way. In this study, *Figure 2* did not indicate a systematic structure.

The question of how to check whether or not the random errors are distributed normally is answered by the histogram and the normal probability plot. These are used to check whether or not it is reasonable to assume that the random errors inherent in the process have been drawn from a normal distribution. The normality assumption is needed for the error rates we are willing to accept when making decisions about the process. If the random errors are not from a normal distribution, incorrect decisions will

be made more or less frequently than the stated confidence levels for our inferences indicate.

The normal probability plot is constructed by plotting the sorted values of the residuals versus the associated theoretical values from the standard normal distribution. Unlike most residual scatter plots, however, a random scatter of points does not indicate that the assumption being checked is met in this case. Instead, if the random errors are normally distributed, the plotted points will lie close to straight line. Distinct curvature or other significant deviations from a straight line indicate that the random errors are probably not normally distributed. A few points that are far off the line suggest that the data has some outliers in it.

The normal probability plot in *Figure 3*. indicated that that it is reasonable to assume that the random errors for these processes are drawn from approximately normal distributions. In this case there is a strong linear relationship between the residuals and the theoretical values from the standard normal distribution. Of course the plots do show that the relationship is not perfectly deterministic (and it never will be), but the linear relationship is still clear. Since none of the points in these plots deviate much from the linear relationship defined by the residuals, it is also reasonable to conclude that there are no outliers in any of these data sets.

The graph of residuals plotted against predicted values and the normal probability plot did not reveal anything particularly troublesome pattern, although the largest positive residual value observed slightly above 3 stands out from the others and the normal plot indicated few points at the extreme. These are not enough in the scattered plot to indicate unsuitability of the model for the study. According to [21] it is possible that a particular treatment combination produces slightly more erratic response than the others. The problem more over is not severe enough to have a dramatic impact on the analysis and conclusions [21]

The normal probability plot helps us determine whether or not it is reasonable to assume that the random errors in a statistical process can be assumed to be drawn from a normal distribution. An advantage of the normal probability plot is that the human eye is very sensitive to deviations from a straight line that might indicate that the errors come from a non-normal distribution. However, when the normal probability plot suggests that the normality assumption may not be reasonable, it does not give us a very good idea what the distribution does look like.

A histogram of the residuals from the fit, on the other hand, can provide a clearer picture of the shape of the distribution. The fact that the histogram provides more general distributional information than does the normal probability plot suggests that it will be harder to discern deviations from normality than with the more specifically-oriented normal probability plot.

The Histogram for this study shown in *Figure 4*. indicated that the histogram is more-or-less bell-shaped, confirming the conclusions from the normal probability plots. One important detail to note about the normal probability plot and the histogram according to [22] is that they provide information on the distribution of the random errors from the process only if

1. the functional part of the model is correctly specified,
2. the standard deviation is constant across the data,
3. there is no drift in the process, and
4. the random errors are independent from one run to the next.

If the other residual plots indicate problems with the model, the normal probability plot and histogram will not be easily interpretable.

Conclusion

A residual analysis procedure was successfully applied to analyze series of similar experiment model for sowing date studies in rice using rice yield as a parameter. The procedure proved to be very simple and easy to implement and it can be applied to any statistical model. The residual analysis showed that the conventional series of similar experiment model can be adequately used to study effect of sowing dates on yield of rice in particular and any other annual crop, generally. The plots verify all questions pertaining to model validity.

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VARIATIONS IN BIRD POPULATIONS IN A BROAD LEAFED WOODLAND: 1975 TO 1998

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Abstract. Variations in passerine populations were examined at a single Common Bird Census location over 24 years. Long term trends in the populations of Robin *Erithacus rubecula*, Blackbird *Turdus merula*, Wren *Troglodytes troglodytes*, Blue Tit *Parus caeruleus* and Great Tit *Parus major* were investigated. The statistical method proposed by Bulmer[1], which overcomes the problem of regressions to the mean, was used to investigate density-dependent variation (DDV). Little evidence of DDV was found and so long-term population trends could be evaluated. The commonest species seen were the Wren and Robin. Significant increases were seen in breeding pairs of Great Tit ($p < 0.001$) and Wren ($p = 0.048$) whereas those of Blackbird decreased ($p = 0.006$) and there was no significant change in breeding pairs of Blue Tit ($p = 0.090$) or Robin ($p = 0.871$). Selection pressures on the populations studied were probably reduced by the consistently mild winters and feeding at bird tables in the gardens near the study area in winter. The CBC technique is recognised as the most accurate method of estimating the size of breeding bird populations. This analysis shows that the CBC data collection method, combined with relatively simple statistical techniques, can be used to accurately interpret local trends in breeding bird populations.

Keywords: *Bird Census, density-dependent variation*

Introduction

In 1962 the British Trust for Ornithology (BTO) instigated the Common Bird Census (CBC) to monitor trends in bird populations[2]. National analyses may mask trends within local populations, including density-dependent variation (DDV). Since many CBC sites have been surveyed for over 20 years they are ideal for investigating local variations within breeding bird populations. This analysis evaluated variations in passerine populations at one such CBC location in Hampshire, UK.

Materials and methods

Southampton Common (147 ha) lies within the city suburbs and is surrounded by suburban gardens. The 57 ha study area in the Common's northwest corner consisted of open parkland, heathland and mature mixed woodland growing on Bracklesham Beds, a combination of pale yellow sands and dark mottled clay. The Common was used for animal grazing until the 1930s and consequently the ground flora is impoverished, the herb layer dominated by Bracken *Pteridium equilimum* and Bramble *Rubus fruticosus*. Open grassland covered a third of the study area. Clumps of woodland consisted of a canopy dominated by Oak *Quercus robur* and Birch *Betula* spp with an understorey of

Holly *Ilex aquifolium* and Hawthorn *Crataegus monogyna*. In 1975, the study area was isolated from the rest of the Common but over the course of the survey vegetation grew over the boundary paths and the territories of some breeding pairs overlapped adjacent areas of the Common. Management and successional change were small, trees were pruned only when dangerous, grass was cut after the ground flora had seeded, and neither herbicides nor pesticides were used. Nest boxes were not provided and hole-nesting species were restricted to natural tree cavities. The main predators were Sparrowhawk *Accipiter nisus*, Magpie *Pica pica*, Squirrel *Sciurus carolinensis*, and Rat *Rattus norvegicus*. From 1980, a pair of Sparrowhawks nested in or near the study area. Between 1975 and 1990, there were an average of 3 pairs of Magpies within the study areas but this rose to an average of 7.5 pairs between 1991 and 1998. Traps were used to control the rat population.

Measurement error is a problem in any study that uses time trend data but was minimised here as all data were collected and interpreted for the CBC[3] by the same observer (J.R. Simms) who used a consistent observational technique. All data were collected during fine weather either in the morning or, less frequently, early evening. Data were analysed by the BTO[2] to estimate the annual number of singing males. Over the study period 39 species were recorded but this analysis was restricted to those with a mean number of breeding pairs of over 10 per annum: Robin *Erithacus rubecula*, Blackbird *Turdus merula*, Wren *Troglodytes troglodytes*, Blue Tit *Parus caeruleus* and Great Tit *Parus major* (Fig. 1). Temperature (°C), rainfall and snowlie data were obtained from a weather station four miles away on the coast (Fig. 2). Mean winter temperature was calculated for each year by taking the mean of the monthly means for the six month consecutive period between October and March inclusive, calculated as the average of the monthly mean maxima and minim.

Multivariable linear regression was used to explore the relationship between the number of breeding pairs of each species, and the mean winter temperature, mean winter rainfall and mean snowfall[4]. The influence of DDV on the number and change in breeding pairs is difficult to analyse because of regression to the mean. For example, even in a series of random numbers, the correlation between the numbers in the series and the change between consecutive numbers is 0.7. To overcome these problems, the methods described by Bulmer[1] was used to test for DDV. Bulmer suggested that a test for DDV could be calculated in two ways which he described as R and R^* . R is the ratio of V (the sum of squares of log densities about the mean log density) and U (the sum of squares of change in log densities): the smaller the ratio, the greater the difference from the mean. Bulmer suggested that measurement error would decrease R , a problem that was recognised in R^* . R^* is the ratio of W (the sum of log densities about the mean log density multiplied by the change in log densities between the next two log densities in the series) and V . DDV will tend to decrease R^* . Values of $R < 1.0552$ and $R^* < -0.373$ indicate evidence of DDV at the 5% level. The power of R^* is lower than R and consequently false negative results may occur in sample sizes of less than 25. Here values for R and R^* were calculated for each species and the results are shown for comparative purposes. We considered that R^* was a better measure to use within this dataset. Poisson regression was used to investigate trends in breeding pairs over time.

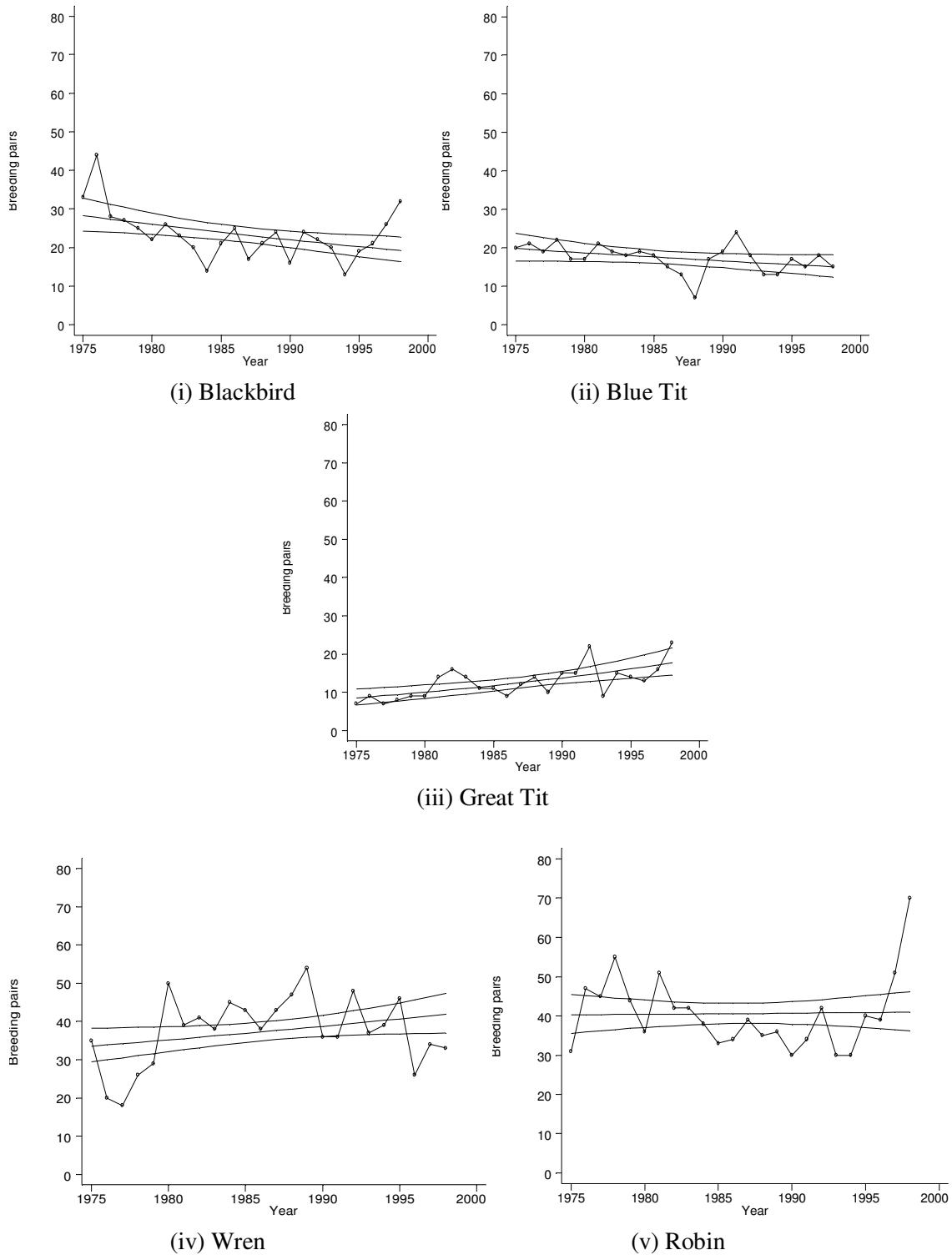


Figure 1. Numbers of singing males, regression coefficient and 95% confidence limits: 1975 to 1998

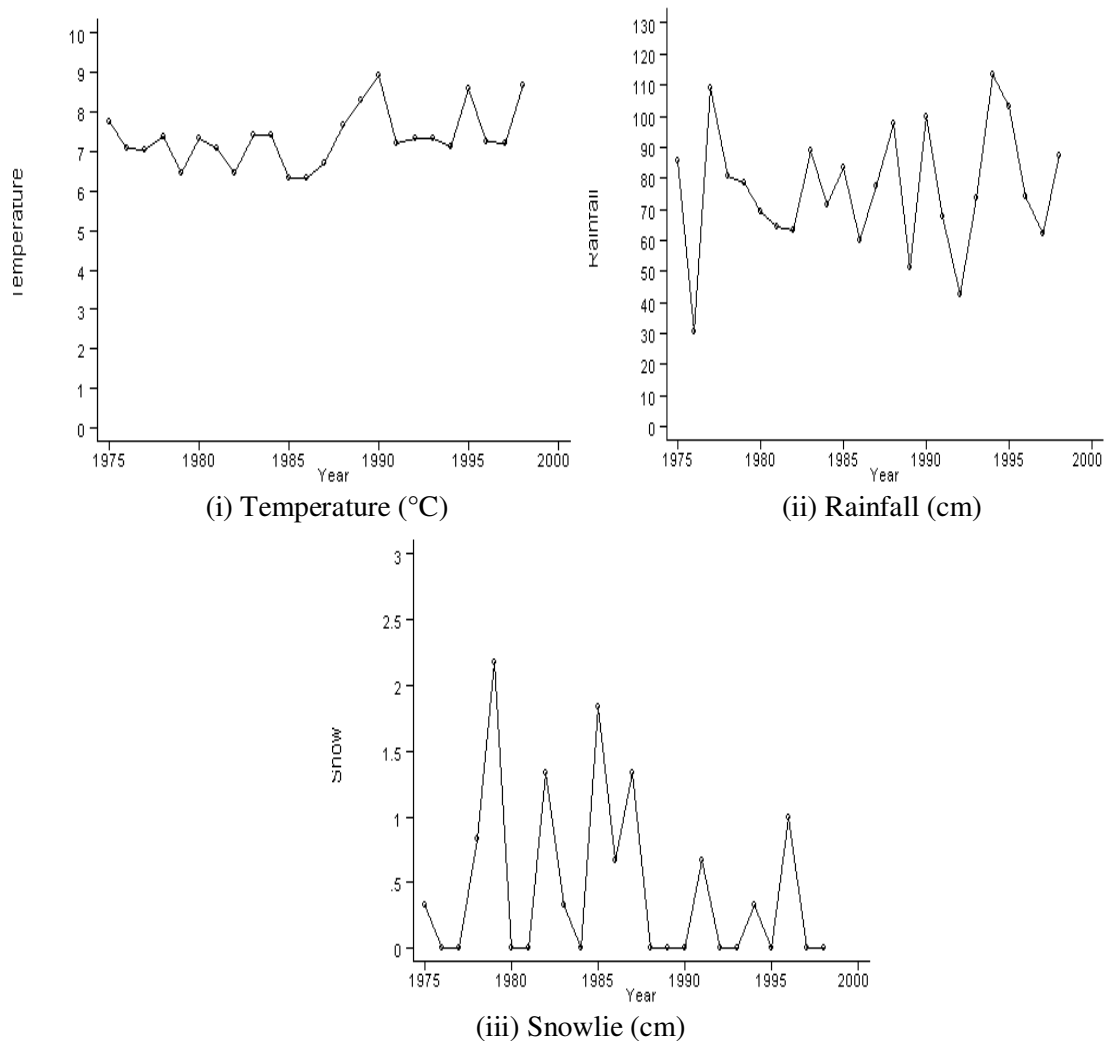


Figure 2. Mean winter temperature (°C), rainfall (cm) and snowfall (cm): 1975 to 1998

Results

Over the 24 years, 853 breeding pairs of Robin (mean per annum 39), 505 of Blackbird (mean/annum = 23), 853 of Wren (mean/annum = 38 pairs), 383 of Blue Tit (mean/annum = 17 pairs) and 263 of Great Tit (mean/annum = 12 pairs) were seen in the study area.

There was no evidence of an association between any of the species studied and the environmental factors using multivariable regression, except between Blackbird and rainfall ($p=0.028$). These results are shown in Table 1. The R values for each species were: Robin (1.045), Blackbird (0.991), Wren (1.035), Blue Tit (0.785) and Great Tit (1.066). The R values for the Robin, Blackbird, Wren and Blue Tit were significant at the 5% level (1.0552). The R^* values were: Robin (-0.088), Blackbird (-0.2551), Wren (-0.2438), Blue Tit (-0.334) and Great Tit (0.0214). These values were not significant at the 5% level (-0.373), although the value for the Blue Tit was bordering on significance.

Breeding pairs of the Great Tits increased by 3.2%, a significant increase over time ($p<0.001$: rate ratio per year =1.032, 95% confidence limit 1.015 to 1.049; a 3.2% increase per year). There was also a significant increase in breeding pairs of the Wren

($p=0.048$: RR 1.009, 95% CL 1.000 to 1.019), whereas there was a significant decrease in the Blackbird ($p=0.006$: RR 0.983, 95% CL 0.971 to 0.995) (Fig. 1). No significant variation was seen for the Blue Tit ($p=0.090$: RR 0.988, 95% CL 0.974 to 1.001) or Robin ($p=0.871$: RR 1.000, 95% CL 0.991 to 1.009).

Table 1. Results of two-way regression analysis

Species	<i>Weather variable</i>					
	Temperature Regression coefficient (per °C of average temp) (95% CL)	<i>p</i> value	Rainfall Regression coefficient (per cm average rainfall) (95% CL)	<i>p</i> value	Snowlie Regression coefficient (per cm average snowlie) (95% CL)	<i>p</i> value
Robin	1.594 (-4.225 to 7.413)	0.576	-0.068 (-0.271 to 0.134)	0.493	-1.174 (-7.566 to 5.217)	0.707
Blackbird	-0.437 (-4.551 to 3.676)	0.828	-0.146 (-0.275 to -0.017)	0.028	-1.029 (-5.512 to 3.452)	0.638
Wren	2.342 (-3.242 to 7.927)	0.394	-0.038 (-0.236 to 0.159)	0.693	-1.945 (-8.099 to 4.207)	0.519
Blue Tit	-0.179 (-2.407 to 2.048)	0.869	-0.056 (-0.130 to 0.017)	0.126	0.157 (-2.280 to 2.594)	0.895
Great Tit	1.875 (-0.614 to 4.364)	0.133	-0.005 (-0.096 to 0.086)	0.910	-1.209 (-4.029 to 1.610)	0.383

Discussion

This study examined variations in passerine populations at a single CBC location over 24 years. No evidence of DDV was found in the Robin, Blackbird and Wren using the R^* statistic and borderline evidence of DDV was seen for the Blue Tit. Other English studies that investigated trends in passerine breeding pairs in similar habitats reported evidence of DDV in the same species as studied here[5], and clutch size, hatching success and territorial behaviour[6]. A national analysis of CBC data also found evidence of DDV in passerines within woodland sites[7]. The fact that DDV was not detected here was not due to a type I statistical error as the tests used were capable to detecting DDV within these types of data. Rather selection pressures on the species studied could have been reduced by feeding at bird tables in the gardens near the Common in winter, and the consistently mild winters of the local maritime climate. Potential sources of bias, including habitat change, predation and the concentration of singing and other conspicuous activities on dry days in wet years[7], are likely to be minimal as they promote DDV.

The absence of DDV allowed an opportunity to evaluate long-term trends in breeding pairs. The commonest species seen were the Wren and Robin. Although there was a considerable fluctuation from year to year, there was a significant increase in the Wren population over the study period in line with national and European trends[8]. Since Wrens do not generally feed at bird tables their populations are sensitive to hard winters[9]. Here this selection pressure was probably reduced by the consistently mild winters. There was no significant variation in the Robin population compared to the overall rise seen in the UK and many parts of Europe[8]. The significant decline in breeding pairs of Blackbirds mirrors the shallow decline seen in the national CBC records since the 1970s. Breeding pairs of Great Tits increased in line with the national

trend seen since the late 1960's, but there was no significant variation in the number of Blue Tits in contrast to the shallow increase seen nationally over the same period[8]. Feeding at bird tables during the winter is known to reduce DDV[10] and Robins, Blackbirds, Blue Tits and Great Tits feed in the gardens near the Common in winter which may explain the increases in breeding pairs. However, the decline in the Blackbird over the same period has been attributed to reduced survival of adults and juveniles[11]. It is likely that other factors which were not measured in this analysis, such as nutrition, clutch size and survival, influenced the populations studied and reduced the sensitivity of the investigation. Whilst their inclusion would have allowed a more detailed exploration of the variation in population density, the study would have been much more time consuming and resource intensive.

The CBC technique is recognised as the most accurate method of estimating the size of breeding bird populations. This analysis shows that this data collection method combined with relatively simple statistical techniques can be used to accurately interpret local trends in the populations of breeding birds.

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TRENDS IN RESEARCH ON THE POSSIBLE EFFECTS OF CLIMATE CHANGE CONCERNING AQUATIC ECOSYSTEMS WITH SPECIAL EMPHASIS ON THE MODELLING APPROACH

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Abstract. Knowledge on the expected effects of climate change on aquatic ecosystems is defined by three ways. On the one hand, long-term observation in the field serves as a basis for the possible changes; on the other hand, the experimental approach may bring valuable pieces of information to the research field. The expected effects of climate change cannot be studied by empirical approach; rather mathematical models are useful tools for this purpose. Within this study, the main findings of field observations and their implications for future were summarized; moreover, the modelling approaches were discussed in a more detailed way. Some models try to describe the variation of physical parameters in a given aquatic habitat, thus our knowledge on their biota is confined to the findings based on our present observations. Others are destined for answering special issues related to the given water body. Complex ecosystem models are the keys of our better understanding of the possible effects of climate change. Basically, these models were not created for testing the influence of global warming, rather focused on the description of a complex system (e. g. a lake) involving environmental variables, nutrients. However, such models are capable of studying climatic changes as well by taking into consideration a large set of environmental variables. Mostly, the outputs are consistent with the assumptions based on the findings in the field. Since synthesized models are rather difficult to handle and require quite large series of data, the authors proposed a more simple modelling approach, which is capable of examining the effects of global warming. This approach includes weather dependent simulation modelling of the seasonal dynamics of aquatic organisms within a simplified framework.

Keywords: *ecological modelling, model system, community ecology, global warming, seasonal dynamics*

Introduction

On the basis of scientific knowledge and observations, it can be stated that global warming has become a reality in the 20th century, which has an anthropogenic origin. Since 1850, global mean temperatures have increased by 0.76 °C. Between the years of 1995 and 2006 (12 years), 11 years ranged within the warmest ones measured since 1850. In order to determine the effects of climate change expected, the emissions data of social-economic developmental stages are used. Results indicated at least 2 °C

increase in temperature in the 21st century, however it can be 5 °C as well (IPCC 2007, a).

Climate change can influence aquatic ecosystems in a very sensitive way. Arid periods can bring marshland into shrivelling, increasing evaporation reduce water surface in ponds and may bring in a low water stage for a good while (Hufnagel et al. 2008). In the latest IPCC report (2007, b), there was evidence of earlier deductions, namely increasing water temperature has a negative effect on the water quality of lakes by reducing the oxygen concentration in the hypolimnion, releasing of phosphorus from the sediment and changing the mixing patterns (McKee et al. 2003; Verburg et al. 2003; Winder & Schindler 2004, Jankowski et al. 2006). Beyond the large scale processes and habitat changes mentioned above, there are many other effects of drastic warming which cannot be explored without deeper understanding of the relationship between aquatic communities and weather conditions (especially temperature).

Essentially, seasonal dynamics is determined by the climate, principally by temperature. The concept of seasonal dynamics has been published in several forms. It has been come out like „seasonal variation”, „seasonal changes” or „seasonal cycle” considering changes in the abundance, biomass or in the composition of assemblage. Generally, seasonal dynamics is the temporal change (individual numbers and composition) of the examined assemblage. Since these processes are influenced by climate (principally temperature), collecting data on seasonal dynamics and exploring its connections with weather conditions (mainly temperature) and also modelling may result in deeper understanding of the effects of climate change.

Our knowledge on the seasonal succession of freshwater (mostly lake) systems is not so deep than those of terrestrial systems. In addition, in aquatic habitats researchers did not identify such distinct successional states than in terrestrial food webs. It is not meaningful to look at the pelagic zone of lake systems as one food web, rather succession of different food webs. Take the so-called PEG (Plankton Ecology Group) model (Sommer et al. 1986) adapted to describe the seasonal succession of freshwater plankton. It distinguishes 24 successional states with different significance of abiotic factors and biotic interactions. This plankton succession model is the first one among models constructed on the basis of synthetizing numerous case studies in that topic; however, it is valid only for temperate deep lakes of definite stratification. There are no more general succession models available for other types of water bodies such as shallow lakes.

Still our knowledge on seasonal succession in aquatic ecosystems has some shortcomings due to the small size of planktonic elements playing a major role in this process. However, plankton communities are in the focus of such research owing to their small size and high reproduction rate, thus they respond to indirect effects within a short period as well (Straile 2005). To illustrate this point take e. g. the r-strategic centric diatoms (Centrales) in the River Danube, which are able to proliferate within a day resulting in rapid multiplication, thus, high regeneration of phytoplankton after flood events (Kiss 1986).

Since the phytoplankton plays a crucial role in climate control, investigations of such communities are of major importance in relation to global warming (Charlson et al. 1987; Williamson & Gribbin 1991). Due to the high biomass and photosynthetic activity, phytoplankton generates half of the oxygen quantity needed for human life; moreover by fixing carbon-dioxide it has a major contribution to climate control (Duinker & Wefer 1994).

There are some more reasons supporting the significance of such plankton surveys. Since the last glacial maximum, variation in environmental variables based on the variation of planktonic communities in lake systems within a year seems to be analogous with climate change in the past (Reynolds 1997). There is strong evidence for systematic changes in plankton abundance and community structure over recent decades in many areas worldwide. Ongoing plankton monitoring programmes worldwide (some have now been running for more than 50 years, e. g. CalCOFI in California, Odate in Japan, Continuous Plankton Recorder in the North Atlantic) will act as sentinels to identify future changes in marine ecosystems (Hays et al. 2005). It has been shown that abundance and structure of the zooplankton community display considerable variations on seasonal, interannual, and regional scales (Arashkevich et al. 2002). Predictable patterns of biodiversity often occur in freshwater pelagic communities over yearly cycles in temperate regions of the globe (Bernot et al. 2004).

Several studies draw attention to the negative effects of climate change on fish (Rahel et al. 1996; Schindler 1997; Stefan et al. 2001). Many studies are run on the - mostly harmful - consequences of climate change on the water quality of freshwater ecosystems, habitat of fish, fish communities and fishery exploitation. In the recent past, a review was published on this topic (Ficke et al. 2007).

There have been some articles summarizing our knowledge on the possible effects of climate change on aquatic habitats in general (Carpenter et al. 1992, Schindler 1997). The Pew Center (Poff et al. 2002) has summarized such results for the U.S. by presenting several case studies for all types of freshwater and marine ecosystems. Most reviews – significant in the sense of community ecology as well – discuss limnobiological aspects. Straile (2005) gives a detailed overview of our recent knowledge on the effects of climatic factors on the food web and seasonal dynamics of lakes in the temperate zone, discussing also the possible effects of climate change - primarily focusing on plankton communities.

According to the database of Web of Science, there was a total of 45209 articles published dealing with climate change until February 2009, from which 3.7 % concerns biodiversity and/or ecology (*Table 1.*). In a wider sense, 8.6 % of articles discuss ecosystem-related questions. Despite the large proportion of water bodies (24.6 %) including lakes (6.9 %) and rivers (7.6 %), studies on their ecosystems add up to 0.6 % only. When focusing on articles having relevancy in community ecology (which add up to 0.6 % only without closer restrictions), there are few of them concerning freshwater systems. This indicates that there is a need for putting emphasis on the research of climate change in relation to community ecology in water systems as well.

From the point of view of exploitation, research on fish has been a thoroughly investigated field among hydrobiological surveys, also in relation to climate change. Beyond doubt, the significance of phytoplankton in climate change research is of major importance, which is confirmed by the large number of publications. However, freshwater habitats are underrepresented, what is more, lakes are favoured over rivers. Nevertheless, more studies are run on the relationship between rivers and climate change as compared to that of lakes. Such features can be seen in modelling studies as well. Among climate change research, the modelling approach has been strongly adopted even by aquatic surveys. Rivers have been the objects of modelling more regularly than lakes, which is in accordance with the fact, that rivers are expected to respond to climate change in a very sensitive way. However, phytoplankton modelling studies have rarely focused on rivers. All in all, these findings draw attention to the

significance of rivers as far as climate change is concerned; however, their ecological features remained relatively poorly-known.

Table 1. Number of publications dealing with climate change and their proportion in % based on different combinations of key words in the database of Web of Science

Keywords	pc	%
Climate change	45209	100,0%
Climate change AND biodiversity	1654	3,7%
Climate change AND ecology	1654	3,7%
Climate change AND ecosystem	3878	8,6%
Climate change AND water	11104	24,6%
Climate change AND lake	3112	6,9%
Climate change AND ocean	5897	13,0%
Climate change AND river	3421	7,6%
Climate change AND lake ecosystem	267	0,6%
Climate change AND river ecosystem	254	0,6%
Climate change AND aquatic ecosystem	155	0,3%
Climate change AND wetland 495	495	1,1%
Climate change AND ecology AND community	290	0,6%
Climate change AND aquatic community	89	0,2%
Climate change AND aquatic food web	31	0,1%
Climate change AND lake AND arctic	286	0,6%
Climate change AND lake AND subarctic	46	0,1%
Climate change AND lake AND temperate	176	0,4%
Climate change AND lake AND tropic	2	0,0%
Climate change AND zooplankton	330	0,7%
Climate change AND phytoplankton	704	1,6%
Climate change AND phytoplankton AND lake	188	0,4%
Climate change AND phytoplankton AND river	67	0,1%
Climate change AND fish	898	2,0%
Climate change AND fish AND lake	157	0,3%
Climate change AND fish AND river	148	0,3%
Climate change AND model	13964	30,9%
Climate change AND model AND water	4281	9,5%
Climate change AND model AND lake	638	1,4%
Climate change AND model AND river	1297	2,9%
Climate change AND wetland AND model	137	0,3%
Climate change AND phytoplankton AND model	191	0,4%
Climate change AND phytoplankton AND model AND lake	37	0,1%
Climate change AND phytoplankton AND model AND river	19	0,0%

On the basis of the reviews the possible effects of climate change have been investigated by four approaches:

- One possible tool is based on long-term field monitoring and observation of climatic events in the last decades; it describes the tendency attributable to climate change in aquatic ecosystems. Such findings are the basis for making predictions to the possible future changes. In the future, a more drastic climate change is expected as compared to the recent past, which may bring more significant effects and may create several unexpected processes.
- Other works forecast possible future scenarios by examining the connections between paleolimnological fossils and climate change in the past (Quinlan et al. 2005, Larocque et al. 2001). Research on lake sediments is particularly capable of supporting evidence for the effects of climate change on lake systems in the past (Cohen, 2003). Nevertheless, such findings are limited and it is questionable whether they are capable of understanding the relationship between certain coenological processes and climate change. On the other hand, future climate change affects waters harmed by human activity, thus the outcome may be somewhat different than in the past.
- One more approach includes microcosm (e. g. McKee et al. 2002, Peperzak 2003) and mesocosm (e. g. Nougulier et al. 2007, Christoffersen et al. 2006, McKee & Atkinson 2000) experiments, which are able to simulate the global warming within a long-term experiment under laboratory or at least regulated conditions. Thus, within an experimental design, it is possible to study the reaction of the assemblage on warming. Present approach is particularly suitable for the interpretation of certain phenomena and for hypothesis testing. On the basis of the experiments there is a potential to form new hypotheses and set up mathematical models (e. g. De Senerpont Domis et al. 2007).
- Finally, the modelling approach is a useful tool to make predictions for the future (as long as the assumptions are fulfilled), that we cannot achieve either by means of simulation experiments or field work.

The topic is rather diversified, so it would mean an enormous effort to review all stream thoroughly. It is inevitable to have a look at the observations on the climate change in the recent past and these days, thus we can make predictions of the possible effects and trends of climate change in the future. However, present study is not destined for detailed description of issues such as paleoecological research and experimental approach, because these topics have grounds of their own. Research on the possible effects of climate change is typically the topic that cannot be investigated by empirical approach; even experiments may result in formulating new hypotheses or focusing on minor issues only. Thus, here, the modelling approach will be discussed in a more detailed way.

Observed effects of climate change and implications for the future

General statements for temperate freshwater systems

Rising water temperatures induce direct physiological effects on aquatic organisms through their physiological tolerance. This, mostly species-specific effect can be demonstrated through the examples of two fish species, the eurythermal carp (*Cyprinus carpio*) and the stenothermal *Salvenius alpinus* (Ficke et al. 2007). Physiological

processes, such as growth, reproduction and activity of fish are affected by temperature directly (Schmidt-Nielsen 1990). Species may react on changed environmental conditions by migration or acclimatization. Endemic species, species of fragmented habitats, and systems with east-west orientation are less able to follow the drastic habitat changes due to global warming (Ficke et al. 2007). At the same time, invasive species may spread, which are rather able to tolerate the changed hydrological conditions (Baltz & Moyle 1993).

What is more, global warming induces further changes in physical and chemical characteristics of water bodies. Such indirect effects include decrease in dissolved oxygen content (DO), change in toxicity (mostly increasing levels), trophic status (mostly indicating eutrophication) and thermal stratification.

DO content is related to water temperature. Oxygen gets into water through diffusion (e. g. stirring up mechanism by wind) and photosynthesis. Plant, animal and microbial respiration decrease the content of DO, particularly at night when photosynthesis based oxygen production does not work. When oxygen concentration decreases below 2-3 mg/l we have to face the hypoxia. There is an inverse relationship between water temperature and oxygen solubility. Increasing temperatures induce decreasing content of DO whereas the biological oxygen demand (BOD) increases (Kalff 2000), thus posing a double negative effect on aquatic organisms in most systems. In the side arms of eutrophic rivers, the natural process of phytoplankton production-decomposition has an unfavourable effect as well. Case studies of the side arms in the area of Szigetköz and Gemenc also draw attention to this phenomenon: high biomass of phytoplankton caused oxygen depletion in the deeper layers and oversaturation in the surface (Kiss et al. 2007).

Several experiments were run on the effects of temperature on toxicity. In general, temperature dependent toxicity decreases in time (Nussey et al. 1996). On the other hand, toxicity of pollutants increases with rising temperatures (Murty 1986.b), moreover there is a positive correlation between rising temperatures and the rate at which toxic pollutants are taken up (Murty 1986.a). Metabolism of poikilotherm organisms such as fish increases with increasing temperatures, which enhances the disposal of toxic elements indirectly (MacLeod & Pessah 1993). Nevertheless, the accumulation of toxic elements is enhanced in aquatic organisms with rising temperatures (Köck et al. 1996). All things considered, rising temperatures cause increasing toxicity of pollutants.

Particularly in lentic waters, global warming has an essential effect on trophic state and primary production of inland waters through increasing the water temperature and changing the stratification patterns (Lofgren 2002). Bacterial metabolism, rate of nutrient cycle and increase in algal abundance are increasing with rising temperatures (Klapper 1991). Generally, climate change related to pollution of human origin enhances eutrophication processes (Klapper 1991; Adrian et al. 1995). On the other hand, there is a reverse effect of climate change inasmuch as enhancement of stratification (in time as well) may result in concentration of nutrients into the hypolimnion, where they are no longer available for primary production (Magnuson 2002). The latter phenomenon is only valid for deep, stratified lakes with distinct layers of aphotic and tropholitic.

According to the predictions of global circulation models climate change is more than rise in temperatures purely. The seasonal patterns of precipitation and related flooding will also change. Frequency of extreme weather conditions may intensify in water systems as well (Magnuson 2002). Populations of aquatic organisms are

susceptible to the frequency, duration and timing of extreme precipitation events including also extreme dry or wet episodes. Drought and elongation of arid periods may cause changes in species composition and harm several populations (Matthews & Marsh-Matthews 2003). Seasonal changes in melting of the snow influence the physical behaviour of rivers resulting in changed reproduction periods of several aquatic organisms (Poff et al. 2002). Due to melting of ice rising sea levels may affect communities of river estuaries in a negative way causing increased erosion (Wood et al. 2002). What is more, sea-water flow into rivers may increase because of rising sea levels; also drought contributes to this process causing decreased current velocities in the river.

Climate change may enhance UV radiation. UV-B radiation can influence the survival of primary producers and biological availability of dissolved organic carbon (DOC). The interaction between acidification or pollution, UV-B penetration and eutrophication has been little studied and is expected to have significant impacts on lake systems (Magnuson 2002; Allan et al. 2002).

Observed and expected reactions of aquatic communities

Research on the relationship between climate change observed in the previous decades and aquatic food webs (Schindler et al. 1990, Livingstone 2003, Straile et al. 2003) is the key for getting a picture of the possible effects of global warming. There has been evidence for the effect of global warming on aquatic (particularly planktonic) communities in lakes, which was related to changes of some environmental parameters such as nutrient loading (Annaville et al. 2002; 2007; Molinero et al. 2006). The central regulatory role of climate in structuring the zooplankton community was demonstrated through the examples of European shallow lakes on the basis of surveys performed in 81 lakes (Gyllsrröm et al. 2008). Pelagic plankton is the major dependent of climate change, benthic and periphytic taxa are rather influenced by macrophytes. Some studies put emphasis on specific aspects of climate change e. g. the duration of ice cover or the duration of spring full circulation (Rolinski et al. 2007), others stress the influence of temperature in a more general way (Wagner & Benndorf 2006). Further characteristic trends in research of climate change in lakes are examination of the variation in duration of ice cover, spring phytoplankton blooms, clear water period, fish abundance and predator-prey interactions (Magnuson et al. 1990; Schindler et al. 1990; Scheffer et al. 2001; Winder & Schindler 2004; Blenckner 2005; Mooij et al. 2005).

Climate change can affect aquatic food webs in many ways. It can alter duration and timing of the successional status (Straile 2002), or modify the structure of the food web within different successional stages (Wehenmeyer et al. 1999). In the life-history of certain species there may be some changes as well: timing of reproduction period of long-lived species or timing of metamorphosis by copepods, shifting of diapause towards earlier or later dates all may cause changes in the structure of the whole food web (Straile 2005).

Faster and more drastic warming of water bodies would result in earlier phytoplankton blooms implicitly. Several case studies forecast earlier peaks of algal biomass, which goes hand in hand with increased biomass (Flanagan et al. 2003), particularly in the winter term (Thackeray et al. 2008). Invasive cyanobacteria such as *Cylindrospermopsis raciborskii* playing a central role in blooms showed evidence for earlier population peaks (Wiedner et al. 2007). Peperzak (2003) found that four harmful algal species doubled their growth rates at a 4 °C temperature rise whereas two harmful

species died rapidly within a microcosm experiment. Two non-harmful species did not significantly change from present to 2100-surface conditions. Christoffersen et al. (2006) conducted experiments in artificial ponds to evaluate the potential effects of global warming on picoplankton and nanoplankton populations. Their results demonstrated that the direct effects of warming were far less important than the nutrient effect, and these variables displayed complex interaction.

Lake food webs are characterized by professed seasonal dynamics, so the effect of climate is strongly dependent on season. Thus, we should not neglect the winter term - often neglected due to the lower biological activity - to include in the study having particular importance in temperate lakes (global warming observed in the 20th century is strongly manifested in the weather conditions of the winter term). In response to that the duration of ice cover period (Adrian et al. 1999, Weyhenmeyer 1999), and also timing and intensity of winter mixing caused by wind (Geadke et al. 1998), which has a significant effect on spring phytoplankton bloom formation, vary in lakes.

In the winter term, mixing period plays a crucial role in determining posterior peaks of phytoplankton production, thus investigation of that period has a major importance in climate change research. Mixing periods in sea and deep lakes are the analogies of barren land (Reynolds 1997). During the winter mixing period, primary production is limited by low temperatures, yearly minimum of incident radiation and yearly maximum of reflection from the water surface. As soon as these assumptions change during the year (increasing temperatures, increasing angle of incidence of solar radiation etc.) phytoplankton production begins to rise. Hence, the understanding and prediction the origin of phytoplankton blooms is of major importance, in this way we may get a picture of the possible outcomes of climate change and other environmental changes (Peeters et al. 2007). It has been shown in the side arms and dead arms of the River Danube that phytoplankton of high abundance can evolve in winter during ice cover dominated by species of cold-tolerant Centrales, Cryptophyta and Volvocales (Kiss & Genkal 1993).

Shallow lakes are the objectives of such research more frequently since they are influenced by changing weather conditions more directly (Gerten & Adrian 2001). Taking into consideration transparency as a fundamental factor in shallow lakes and accepting the concept of alternative stable state planes (Scheffer et al. 1993) the macrophytes dominated clear water state and the plankton dominated turbid state can be distinguished. When nutrient load rises towards critical levels there is a possibility for state change. However, phytoplankton dominated lakes can experience clear water state as well due to increased zooplankton grazing in spring mostly (Gulati et al. 1982; Sommer et al. 1986). Climate change is supposed to influence the transparency of shallow lakes in a negative way by destabilizing the macrophytes dominated clear water state and stabilizing the phytoplankton dominated turbid state (Mooij et al. 2005). This negative effect has been demonstrated through the examples of shallow lakes in the Netherlands and it is based on several findings. Increase in winter precipitation and extreme precipitation events increase the phosphorus load of lakes (Mooij et al. 2005). Rising temperatures cause inner nutrient increase (Jensen & Andersen 1992; Liikanen et al. 2002). Rising temperatures increase mismatch of spring phytoplankton and zooplankton peaks owing to declined predation pressure of zooplankton. Moreover, high temperatures favour cyanobacteria meaning a less efficient nutrient for zooplankton (Dawidowicz et al. 1998; Gliwicz & Lampert 1990). At the same time, predation pressure on zooplankton may increase because of decreasing winter mortality

of fish (Mooij 1996; Mehner et al. 1995; Mooij & Van Nes 1995; Nyberg et al. 2001). Increased rate of sediment resuspension is expected caused by wind (Schelske et al. 1995). Essentially the above-mentioned findings are based on case studies of shallow lakes in the Netherlands, however, Jeppensen et al. (2003) found similar characteristics in Danish shallow lakes implying that climate change creates eutrophication-like effects.

From the point of view of zones characterized by different fish assemblages in rivers, such as trout, grayling, barbel and bream zones, climate change seems to be a very interesting question. Characterization of rivers and river sections on the basis of such zones has been in the focus of interest for a long time (Fritsch 1872). First, Thienemann (1925) distinguished six zones, then, Huet (1949) tried to correlate them with abiotic factors such as water depth and decline of water. Temperature can be regarded as the major environmental parameter determining the development of fish assemblages and those zones along the river (Flebbe et al. 2006; Pont et al. 2006). Hence, rising water temperatures would result in realignment and shift of those zones. There have been some studies aimed at predicting the distribution of fish assemblages under rising temperatures by considering the biogeographical limiting factors at the present time (Eaton & Scheller 1996; Rahel 2002).

However, a more drastic global warming is expected in the future, which has no signs in the climatic features of the last decades, generating changes in aquatic ecosystems. The significance of the topic is best highlighted by the study performed in Lake Tanganyika demonstrating that the negative effect of climate change can be more important than those of local human activity or overexploitation of fish stock (O'Reilly et al. 2003).

Quantitative predictions for the future: possibilities for modelling

Beyond doubt, the most plausible method for investigating the possible effects of climate change is the modelling approach. In these days, we are in possession of the global quantitative models of climate system (GCM), which – reflecting economic development and emission of pollutants both from optimistic and pessimistic points of view – are based on different emission scenarios. Models of global scale were downscaled for minor regions referred as regional circulation models (RCM). These models are capable of making quantitative predictions for the future climate on the basis of reconstruction of past climatic events relatively exactly. By means of the data series of regional climate models we are able to forecast possible future states of the populations of aquatic organisms. By describing physical changes of different aquatic habitats (e. g. change in temperatures, hydrological regime) hypotheses can be generated about the effects on the biota living in the water, and also we can make quantitative predictions by creating weather condition dependent models for the community or its elements. Nevertheless, in order to measure the possible effects of climate change on a complex aquatic ecosystem exactly, such model systems would be ideal keys that are capable of describing the relationship between communities and between communities and climatic factors quantitatively. However, such general ecosystem model systems are not typical, so we should make do with models of basic elements specified for a given habitat considering the boundary conditions as well.

Modelling of population dynamics of aquatic organisms has a long history. The German Victor Hensen leading the first great expedition on oceanography in 1889

(Hensen 1892) introduced the terminus of plankton (based on the Greek expression of „planktos” ($\pi\lambda\alpha\gamma\kappa\tau\omicron\varsigma$) meaning „wanderer” or „drifter”). It brought into a boom of research on fishing in the beginning of the 20th century. At that time, the dynamics of fish stocks were described by means of mathematical models just as its connection with biological, physical factors and human impact (Cushing 1975; Gulland 1977; Steele 1977). At the same time, modelling of phytoplankton production began based on the studies of Fleming (1939), Ivlev (1945), Riley (1946) and Odum (1956) primarily. One characteristic trend in research included the control of algal blooms by zooplankton, which appeared first in the work of Fleming (1939) who described the temporal dynamics of phytoplankton biomass by the help of a differential equation. Modelling studies have used the Lotka (1925) and Volterra (1926) predator-prey interaction equations in numerous cases so as to describe the trophic connections between phyto- and zooplankton (Segel & Jackson 1972; Dubois 1975; Levin & Segel 1975; Vinogradov & Menshutkin 1977; Mimura & Murray 1978).

Since the first half of the last century, several trends in modelling have evolved along diverse approaches and community ecological hypotheses often focusing on special issues such as understanding the background behind the dynamics of spatially inhomogeneous plankton communities on the basis of a model for predator-prey interaction including few species (Medvinsky et al. 2001).

In these days, two different modelling approaches seem to be relevant concerning aquatic systems (Peeters et al. 2007). One focuses on phytoplankton response on the grounds of minimalist models (Gragnani et al. 1999; Huisman et al. 2002; Huppert et al. 2002). This school focuses on special questions within the topic given. Such models are not in direct connection with field data, nevertheless, aimed at getting insight into essential ecological processes. The other one includes complex ecological models mostly aimed at modelling planktonic organisms and nutrients on the basis of field data taking into consideration physical, chemical and biological factors as well. Basically, these models aim at predicting long-term changes of the environment such as consequences of human impact and effects of climate change. Characteristically, they include a lot of terms meaning biotic components such as different groups of phytoplankton, zooplankton and higher trophic levels and also abiotic components i. e. environmental parameters measured in the field. In addition, they require very deep understanding of the investigated water body. The large number of parameters may cause several difficulties in complex ecological models (Omlin et al. 2001).

It is worthy to separate the models relevant from the point of view of climate change into two major groups:

- Some models use physics-based hydrological models or model systems often associated with chemical factors. Their common characteristics is that the focus is on modelling of the changes in the physical environment, and changes in community is explained through that (e. g. Hostetler & Small 1999; Blenckner et al. 2002; Gooseff et al. 2005; Andersen et al. 2006).
- Another approach includes modelling of quantitative changes of a population or certain elements of a community in a given habitat. Mostly, phytoplankton is the objective of such studies often separated into some clusters e. g. green algae, diatoms, cyanobacteria (Mooij et al. 2007). On the basis of complexity and validity further subcategories can be generated:
 - The first cluster includes simple models often related to a particular question which can be of major interest as well. Such models can also be

relatively complex including several parameters, but the validity is confined to a close interval due to the special object of modelling or relation to a special environment (e. g. Matulla et al. 2007; Hartman et al. 2006; Peeters et al. 2007).

- Models can be combined into model systems or complex ecosystem models including several environmental factors such as nutrients and light, and it can also be combined with physical models (e. g. Elliott et al. 2005; Mooij et al. 2007; Komatsu et al. 2007; Malmaeus & Håkanson 2004; Krivtsov et al. 2001).
- A more ambiguous modelling method is creation of tactical models neglecting several basic determining factors. Although focusing on the essence and neglecting several basic processes still they provide useful information about the operation of the system in general. Special emphasis is put on the predictive applicability rather than biological interpretation of mathematical operations (Hufnagel & Gaál 2005; Sipkay & Hufnagel 2006; 2007; Sipkay et al. 2008).

Modelling of environmental changes

As a result of climate change (e. g. rising temperatures, changing precipitation), models aimed at describing changes in the physical state of freshwaters quantitatively provide a firm basis for the understanding of the trends in ecological changes. Such studies apply internationally recognized GCM-based RCMs in every case. To illustrate this point take the so-called PROBE model (physical model set up for a lake) (Blenckner et al. 2002), which provided knowledge on the effects of climate change on the ecological state of Lake Erken in Sweden. It was tested on 30-year field data successfully and seems to be suited to forecast the physical changes and their degree. Beyond rising water temperatures, changes include shortening of ice cover periods, decrease in total ice cover events and change in the mixing regime. If it comes true, it will result in enhanced nutrient cycle and productivity.

Hostetler & Small (1999) tried to describe general effects of climate change within a physical model for lakes. The paper deals with physical responses (temperature, mixing, ice cover, evaporation) of North American lakes characterizing their spatial patterns quantitatively. The model was run on theoretical lakes of different region, depth and transparency. Results suggested extreme rise in temperatures (in certain cases water temperatures above 30 °C in summer) meaning major disturbance of aquatic ecosystems.

There are models destined for predicting the temperature rises of rivers. Such a model is the numeric river model of Gooseff et al. (2005), which aimed at forecasting the potential influence of global warming on a shallow reservoir's water temperatures (Lower Madison River, Montana, USA). The study draws attention to water temperature as a critical factor for fish, thus rising temperatures may harm fish stocks strongly.

Some studies try to describe environmental changes by means of a model system considering not only rise in temperatures and changing precipitation conditions, but also change in chemical factors within a whole water system. Such one includes Mike 11-TRANS model system (Andersen et al. 2006) the goal of which is the prediction of changes in hydrological and nutrient conditions in the River Gjern and its catchment area. It is based on GCM of A2 climate change scenario (2071-2100 years) downscaled

for the given region. The model system contains „rainfall-runoff” and „statistical nutrient loss” models by the help of which we are able to make quantitative predictions for the changes (%) in hydrological and nutrient conditions expected in the catchment area (including seasonal aspects as well). Results suggest that average annual total nitrogen (TN) export may increase by 7.7 %.

Simple models of special issues

Essentially, the model of Matulla et al. (2007) aimed at modelling environmental changes through the example of fish assemblages in an upper alpine river by modelling rising water temperatures and drawing conclusions on fish assemblages. The speciality of the approach is that the authors created a model for an index characterizing zones from fish faunistical point of view, so the model is capable of generating quantitative data of changes expected in fish assemblages. Due to the fish faunistical data relative to certain zones of river sections, present approach provides a broader picture of the changes expected in the habitat. Study area was selected in accordance with the objectives i. e. zones easy to separate, and this was the River Mur in Austria. Water temperatures were modelled on the basis of air temperatures considering current velocity as well. Temperature and precipitation data of climate change scenarios (for the period 2027-2049) downscaled for the sampling site were used for making predictions for the future. Fish Zone Index (FiZI) (Schmutz et al. 2000) was used for modelling the fish fauna. This index assigns numbers to seven biocoenotical regions (Illies & Botosaneanu 1963), which are the equivalents of the zones roughly. A species-specific index was introduced (FI_{sp}) indicating the preferences of species for river zones. By the help of the FI_{sp} index values of FiZI were calculated, which is the indicator of the species composition. Results suggest a 70 m translocation of fish assemblages owing to rise in water temperatures and change in yearly cycle of current velocity. Such conditions favour cyprinid species over salmonid ones. Hyporhithal zone – equivalent of grayling zone - may be replaced by epipotamal zone – equivalent of barbel zone. As a consequence, species characteristic for the River Mur such as *Thymallus thymallus* or *Hucho hucho* seem to be in danger because of other species coming to the front. The expected changes are rather unfavourable since - among other things - salmonid species are of major interest from the point of view of fishing.

Through considering climatic factors determining spatial patterns of fish assemblages, there have been some models (e. g. Flebbe et al. 2006) using climate change scenarios indicating that the approach presented above is not completely unique.

Due to the difficulties of complex ecosystem models, and minimalist models being too specific, new trends appeared in modelling studies searching for possible ways between them. Peeters et al. (2007) proposed a mechanistic model for phytoplankton, which does not aimed to describe the behaviour of the whole food web within the year, rather focuses on winter and spring periods only, but considers all important factors within this time. It is worth mentioning that within this period, phytoplankton is limited by light primarily, nutrients are of minor importance and food web is less complex. The model was not created for answering climate change related questions, however, it is of major importance in the topic covered due to its dependence on meteorological conditions. The objective of the study was the simulation of phytoplankton production and that of variation in its timing between years. The model was constructed on the basis of samples taken in Upper Lake Constance. The large and deep (average depth

102 m) lake of perialpine origin experienced eutrophication and reoligotrophication in the last century. The lake has been the objective of studies for a long time, thus its biological background is widely known (Bäuerle & Gaedke 1998). The model was constructed on the basis of samples taken between 1979 and 1994. Both meteorological factors (wind speed, wind direction, air temperature, solar radiation, precipitation, ice cover) and biotic factors (mainly phytoplankton and zooplankton data series) were involved in the model. 1-D mechanistic phytoplankton model was combined with 1-D hydrodynamical model. Basically, mechanistic phytoplankton model includes chlorophyll-a production and loss relative to the vertical water column including also a large set of parameters. Several parameters were constant (e. g. respiration rate, maximum specific production rate at 10 °C), thus certain phenomena such as acclimatization and changes in community structure were neglected. However, zooplankton grazing effect was included in the model. Through the 1-D hydrodynamical model temperature stratification was demonstrated successfully. Results suggest that timing of the initial phytoplankton growth in deep monomictic lakes is determined by turbulent diffusion in the water column. Further factors included in the analysis (e. g. photosynthetically active radiation or water temperature) were of minor importance. These findings are of major importance from the point of view of climate change, since variance in mixing dynamics between years is correlated with meteorological conditions. Climate change affecting winter and spring periods may alter the timing of phytoplankton bloom fundamentally. As a consequence, the study draws attention to the significance of winter period of low biological activity.

Model systems of general practicability, ecosystem models

Models of general applicability able to describe different water bodies are entitled to greater interest as compared to models of specific habitats e. g. a lake. These studies are not focusing on special issues within a simplified modelling framework rather combined into a complex model system focusing on essential processes. There are models that were not created for testing the influence of global warming, rather focused on the description of a complex system (e. g. a lake) involving environmental variables, nutrients.

To illustrate this point, take the PCLake ecosystem model developed for shallow lakes, which were applied in the field of eutrophication (Janse et al. 1992; Janse & Liere 1995), but were also applied for studying the effects of climate change later (Mooij et al. 2007). Impacts of temperature rise on biotic and abiotic components were modelled and other consequences (e. g. increase of winter precipitation) of climate change were also examined in shallow lakes in the Netherlands. The model was created for the simulation of nutrient load and food web dynamics on the basis of data series (nutrient, transparency, chlorophyll, vegetation) obtained over 40 shallow lakes. The effects of warming expected were investigated by the help of 4 temperature scenarios. Critical level of nutrient load was defined during eutrophication when clear water state turns into turbid state and during (re)oligotrophication when its contrary can be observed. The complexity of the model is best highlighted by the wide and large set of input parameters. Main input parameters included water inflow, infiltration or seepage rate, nutrient loading (N, P), particulate loading, temperature, light dimension (depth and dimensions of lake), size of the marsh zone, loading history (initial conditions). A total of 14 temperature dependent component were included in the model (*Table 2.*) from

which 6 were abiotic and 8 biotic. Abiotic processes were described with exponential functions, biotic components were introduced with Gauss-curves by defining their temperature optimum. Macrophytes were simulated through two exponential functions as an optimum function. Respiration got higher values of Q_{10} than production.

Table 2. Temperature dependent components and their parameters in the PCLake ecosystem model (Mooij et al. 2007)

Abiotic process	Temperature coefficient c_i	
Denitrification	1,07	
Diffusion	1,02	
Mineralization in sediment or water	1,07	
Nitrification	1,08	
Reaeration	1,024	
Sedimentation	1,01	
Biotic component	Optimum temperature $T_{opt,j}$	Width around optimum temperature $T_{sigma,j}$
Diatoms	18 °C	20 °C
Green algae	25 °C	15 °C
Cyanobacteria	25 °C	12 °C
Zooplankton	25 °C	13 °C
Zoobenthos	25 °C	16 °C
Planktivorous and benthivorous fish	25 °C	10 °C
Piscivorous fish	25 °C	10 °C
Macrophytes	$Q_{10,prod} = 1,2$	$Q_{10,resp} = 2,0$

Four scenarios were used from which the first one served as control, further three calculated with (1) 3 °C rise in temperatures throughout the year, (2) summer, (3) winter temperature rises only.

According to the findings of PCLake ecosystem model and scenarios, global warming has a strongly negative effect on the status of shallow lakes in the temperate region. Several mechanisms will occur together such as increase in loading and decrease in critical nutrient level of eutrophication. As a result of these, lakes investigated are more likely to turn into turbid state. In line with these, basic changes include increased phytoplankton growth rate and increased availability of phosphorus (owing to increased summer mineralization and temperatures). Rising water temperatures favour phytoplankton mainly cyanobacteria. Enhanced dominance of cyanobacteria draws attention to cumulative risks of climate change in itself. Winter temperatures seem to have significant importance as well. Greatest changes were detected at scenarios with warm winter, whereas scenarios with warm summer showed similar outcomes to the control scenario. This phenomenon is enhanced by changes observed in other trophic levels: e. g. abundance of planktonivorous fish is expected to increase at warm periods, which would result in enhanced predation pressure on zooplankton. The study demonstrated that a complex physical-biological ecosystem model can be well adapted to explore the possible effects of climate change and it draws attention to negative aspects of changes (Mooij et al. 2007).

Model predictions show very similar features. The phytoplankton model PROTECH forecasts enhanced spring phytoplankton bloom with increasing dominance of cyanobacteria on the basis of field data and RCMs downscaled to the region of Lake Bassenthwaite (Elliot et al. 2005). However, summer bloom is expected to disappear

more rapidly due to the nutrient limitation explained by enhanced spring phytoplankton production, thus total productivity will not change.

The majority of ecosystem models are applied to small shallow lakes. As far as large lakes are concerned - ranging from shallow eutrophic to deep oligotrophic ones -, fewer studies can be found (Blenckner 2008). Large lakes response to external effects more slowly due to increased water residence time (Tilzer & Bossard 1992), thus the influence of climate change cannot be compared with those of small lakes at all points.

The mechanistic phosphorus model (LEEDS) of different Swedish lakes combined with a physical lake model is a good example of complex models with wider applicability (Malmaeus & Håkanson 2004; Malmaeus et al. 2005). In order to predict future states regional climate change scenarios were used. Simulations showed smaller Lake Erken to be more reactive to warming - due to increased dissolved phosphorus concentration in spring - than Lake Mälaren (third largest lake in Sweden). These findings support evidence for significance of inner processes. Phytoplankton biomass of Lake Erken is phosphorus limited. These results are true for other similar lakes with increased water residence time pointing out that through climate change eutrophication may cause serious problems in several habitats. Also further examples can be found for models of wider applicability. Such models are LakeWeb (Håkanson & Boulion 2002) and Rostherme models (Krivtsov et al. 2001).

Some papers draw attention to lack of process-based models (Blenckner 2008; Komatsu et al. 2007) including models with (temporal) dynamics of interactions between defined entities (Minns 1992). However, these models can be the keys of prediction contrary to statistical models, which are only valid within a current range (Klepper 1997). The water temperature-ecosystem model WT-ECO (Hosomi et al. 1996) can be regarded as a model of that kind, however, it does not apply GCM scenarios, only calculates with 2-4 °C rise in air temperatures. Although applying climate change scenarios, the LEM model (mathematical eutrophication model) of Hassen et al. (1998) is not able to describe long-term effects of climate change. Its input parameters include atmospheric, chemical and hydrological factors distributed vertically, output parameters are phytoplankton growth rate and dissolved oxygen concentration.

A complex model system was developed in order to examine the long-term effects of climate change on water quality in Shimajigawa reservoir, Japan (Komatsu et al. 2006; 2007). Expected future climate were described on the grounds of A2 GCM based regional climate models for the period 2091-2100, taking the period of 1991-2001 as control. The model system consists of two major parts. The first one is a complex water quality model including Flow and Transport Model, Water-Sediment Model and Ecological Model from which the latter one is based on several parameters. The second part is the Runoff Model considering inflow, precipitation and outflow. The large number of components and connections between them (*Figure 1*) indicate that the water body has been thoroughly studied and monitored for a long time.

The model forecasts increase in trophic status with rising temperatures providing one more example for the fact that climate change may induce negative processes in aquatic ecosystems through increased phytoplankton production primarily. Temperature related processes altogether would result in a more strongly eutrophicated state. Such processes include elongation of thermally stratified periods, increased oxygen demand of aerobic decomposition, facilitated phosphorus release from the sediment and its increased concentration in the hypolimnion.

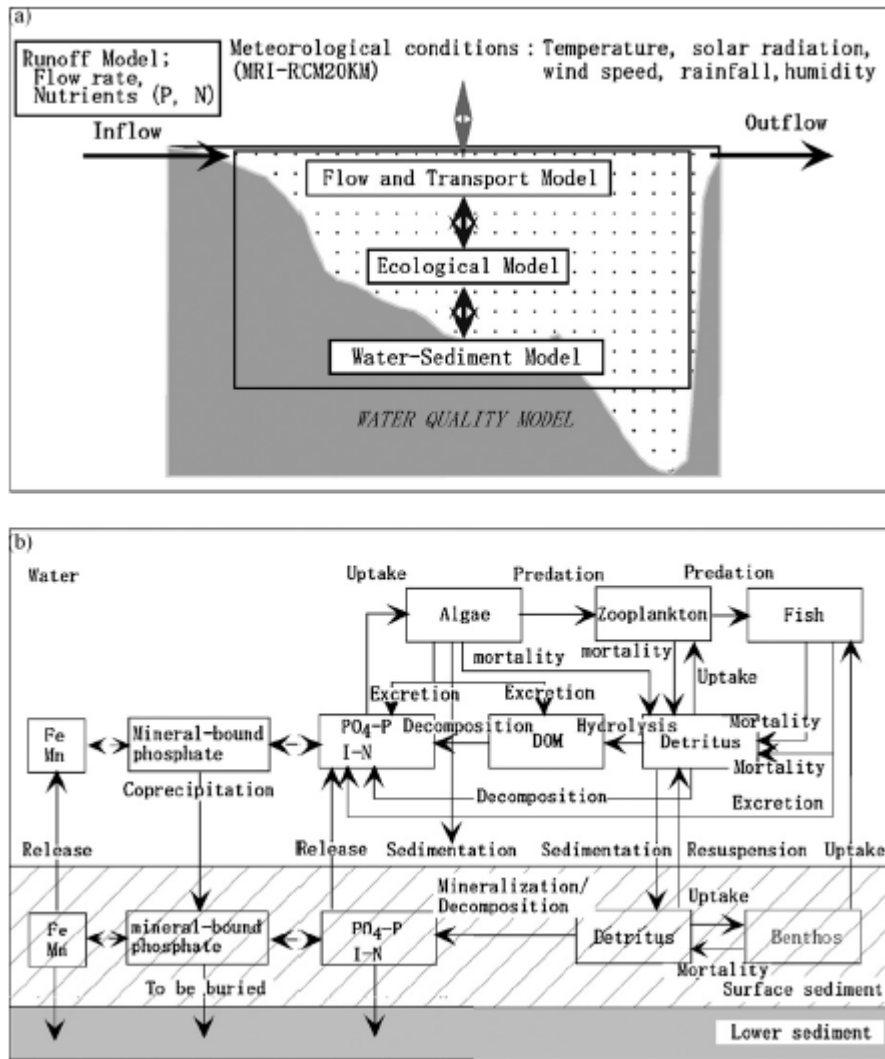


Figure 1. Components of the water quality model combined with the runoff model developed for a West Japanese reservoir (a), compartments and connections of its integrated ecological model (b) (Komatsu et al. 2007)

The above-mentioned examples showed evidence of the capability of ecosystem models to define the effects of rising temperatures on aquatic freshwater systems in a detailed way through describing basic connections quantitatively. However, the applicability of such models to other water bodies presents some difficulty even within similar habitats. Biggest problem one has to face is lack of data and their availability (Porter et al. 2005). On the one hand, data already presented is not easy to obtain, on the other hand, long-term data sets are not achieved on the basis of the same methodology in each cases. What is more, complex models require data often not available regarding several water bodies. In most cases, this is why model systems describing general phenomena cannot be adapted to several habitats of major importance.

Possibilities of tactical modelling (i. e. simplified modelling of seasonal dynamics)

As mentioned above, there are numerous approaches so as to describe the trends in change of physical, chemical features, population or community components of freshwaters under climate change by means of model construction. Some models consider the habitat (lake, river) as a physical unit and try to describe the change in physical parameters, thus we can make statements of communities living in the water on the basis of our recent knowledge. Certain models focus on special issues of a defined water body, which can be very useful from the point of view of the given question, but examination of their methodological approach provides possibilities of applicability to other issues or habitats. Complex ecosystem models incorporating most basic processes of freshwater systems may be the keys of the comprehensive understanding of the effects of climate change. However, modelling methodology of climate change related research cannot be regarded as adequate yet. The root of the problem is lack of synthesizing models and their practicability. These models require quite a lot of information of which we are not familiar with definitely. On the other hand, often we manage to set up a complex model, but its parameters cannot be determined due to lack of field data. Thus, instead of such strategic models often tactical ones are used, which focus on the essence and may neglect some important pieces of information at the same time. Still they can be useful tools to understand the general functioning of the system. This is achieved through stressing the factor regarded as the most crucial and neglecting the other processes. It raises the question whether it is meaningful to stress one factor as the most important one, on the basis of which the seasonal dynamics of the assemblage investigated can be described. Several authors draw attention to the central regulatory role of temperature. According to Christou & Moraitou-Apostolopoulou (1995) temperature is the main factor accountable for the temporal variation of mesozooplankton. Iguchi (2004) and Dippner et al. (2000) got to similar results. Temperature correlated positively or negatively with almost each copepod species depending on the ecological need of the species given (Christou, 1998). Long-term changes in densities of numerous species of Copepoda were related to temperature and salinity (Viitasalo et al., 1990; Meise-Munns et al., 1990; Baranovic et al., 1993). Consequently, stressing temperature as the most important regulating factor seems to be relevant. By taking into consideration the failures and assumptions of the model it is possible to explore the effects of climate change most likely to occur (Hufnagel & Gaál 2005). In order to model the seasonal dynamics of population collectives model system already presented can be used (Ladányi et al. 2003). When using long-term data to construct models, predictive applicability has priority over biological interpretation of mathematical operations through stressing one regulating factor as the most essential and neglecting the other ones. Emphasizing temperature as the most important factor seems to be obvious when modelling seasonal dynamics. In this case, the model hypothesizes temperature to be the only regulating factor, thus the pattern is determined by the daily temperatures, other effects (e. g. trophic connections and other interactions between populations) may appear within this term or hidden.

On the basis of the above-mentioned conception, a rather simple mathematical model can be created of which the essence is: daily abundance can be calculated by multiplying abundance of the previous day by a temperature function (Hufnagel & Gaál 2005; Sipkay & Hufnagel 2006; 2007; Sipkay et al. 2007, 2008.a-b). Running the model with the data series of different climate change scenarios we get a picture of future abundance of the investigated species, which should be handled watchfully. The goal of

the approach is not prediction, rather the comparative assessment of different climate change scenarios within the framework of a realistic model situation.

The objects of modelling can be a group of high abundance playing a major role in the aquatic food web (e. g. total zooplankton abundance), and also abundance or biomass of a dominant species (Vadadi et al. 2007). Another approach includes modelling the seasonal dynamics of some coenological index. Such approach was proposed by Sipkay & Hufnagel (2007) through a case study performed in Lake Balaton. The authors introduced the „Coenological Intensity Index” (CII) characterizing the macroinvertebrate assemblage as a unit. This index considers abundances, biovolume and Shannon diversity simultaneously and it was the object of modelling as well.

In the simplest case, the multiplier describing temperature dependence is determined by constants related to temperature intervals (Sipkay & Hufnagel, 2006; Vadadi et al. 2007). These constant values are the model parameters, which can be optimized with the MS Excel Solver program when fitting the data. However, instead of temperature intervals being often distributed in a complex way, we supposed that the temperature reaction-curve must be the sum of optimum-curves, since temperature optimum-curves of the different developmental stages of the species in question or its distinct subpopulations can be summed. Thus, the values of the multiplier are determined by a continuous function (sum of normal distributions) depending on the daily temperatures.

Experimental ponds are the best objects for describing and modelling the connection between weather conditions and seasonal dynamics in a more exact way through minimizing the number of external regulating factors. This means a transitional approach between laboratory experiments and field observations. Such experiments have been conducted in an artificial pond in Budapest, Hungary. Phytoplankton, zooplankton and macroinvertebrates have been sampled quantitatively and semiquantitatively at regular frequencies of one or two weeks. Through this sampling design (sampling different assemblages regularly in a harmonized way) the food web model can also be constructed. First attempt of that was a model in which phytoplankton abundance was determined by temperatures, and abundances of zooplankton groups and that of a dominant macroinvertebrate species (Vadadi et al. 2008). Even if we do not understand exactly the reason for temperature being the most important regulating factor, still we can make predictions for the periods 2050 and 2070-2100 respectively as regards abundances by running the model with the data series of climate change scenarios. Results suggest that climate change will influence algae and zooplankton in a similar way i. e. decreasing abundances, abundance peaks will occur earlier within years.

One failure of the models illustrated above may be that they are not reliable adequately due to the relatively short-term data series available for fitting. However, the approach mentioned above was applied to long-term data series of the Hungarian Danube Research Station (at Göd) including the period of 1981-1994. First, a model was created describing the seasonal dynamics of a copepod species of high abundance being presented throughout the year on the basis of the above-mentioned database (Sipkay et al. 2008). Results indicated 1-1.5 month shift in the abundance peak towards earlier dates.

Seasonal dynamics of phytoplankton in the River Danube was described by means of a more complex model (Sipkay et al. 2008.b). A detailed (taxa were identified to species level), long-term (1983-1996) database with sampling frequency of weekly intervals is

available for the phytoplankton of the River Danube at the area of Göd. In addition, the temperature dependence of the phytoplankton group located on the basal level of food web is more direct and the number of further regulating factors are less than those of zooplankton and macroinvertebrates. Phytoplankton is not limited by nutrients, rather temperatures and light play a major role in determining seasonal dynamics of algae in the River Danube (Kiss 1994). Since this being the case, it is necessary to include these regulating factors into the phytoplankton model. The model is the linear combination of the temperature optimum-curves of 12 theoretical species. These 12 theoretical species characterized by different temperature optimums and tolerance intervals can multiply at various rates. However, the model includes not only a temperature dependent multiplier but also a light dependent one (through a minimum function), thus the multiplier with lower values counts in each case. Light dependent growth rate involves the environment's carrying capacity meaning a top-down limit for phytoplankton. It can be described with a sinus curve of which the parameters were adjusted considering all-time minimum and maximum abundances of phytoplankton, and order of magnitude of difference between winter and summer maximum values. This design represents differences in lighting periods during the year. Based on the simulation of phytoplankton assemblages in the River Danube, average plankton production is not likely to alter significantly, but on the whole, rather increasing abundance is expected as a consequence of climate change. However, between years variability will increase drastically and significantly.

Summary, discussion

Similarly to ecological surveys, research on the effects of climate change on freshwater ecosystems can be divided into three methodological approaches. First, we have the possibility to explore the influence of climate change in the past by means of field observations. Fundamentally, paleoecological surveys also range within the above-mentioned stream. Secondly, particularly regarding some minor phenomena, experiments under controlled conditions can shed some more light on the possible effects of climate change. Thirdly, model construction includes creating exact mathematical description (mathematical model) of the simplest theory of the phenomenon in question by means of basic biological phenomena and hypotheses. The kernel of the approach can be described by a logical cycle including testing (contrasting with case studies) and improving (development and fitting) of the model. By the help of this methodology, we get a more and more realistic theory of the investigated phenomenon, however, at an early stage we only use some parts of information available.

Each approach mentioned above has its advantages and disadvantages. Reliable, watchfully overseen, certified knowledge can be provided by experiments. When the research objective is a rather complex phenomenon, having importance in application as well, observation and detailed description of it is required in order to formulate realistic hypotheses. Field research in ecology with complex approach is constrained to present single data and correlation between some variables, otherwise such complex hypotheses would be formulated that it would be almost impossible to test them. In order to open up the reasons behind complex phenomena it is essential to formulate hypotheses within simulation models, because in absence of that it would be impossible to choose between alternative hypotheses. Another advantage of the simulation method is that it may point

out misleading interpretation, which has the potential to become a problem both during data analysis (approach 1) and when combining the results of experiments (approach 2).

The main potentials of modelling were reviewed ranging from the physical environment to aquatic communities and their elements. The latter provides relatively exact predictions for future changes in aquatic systems by the help of climate change scenarios. There are complex ecosystem models that were not created for testing the influence of global warming, however, by taking into consideration a large set of environmental variables, they are capable of studying climatic changes as well. Mostly, the outputs are consistent with the physical changes (change in ice cover and mixing patterns) expected. To recap what has been reviewed, global warming may create eutrophication-like features in the water bodies of the temperate zone, particularly in lakes, which has the potential to become a very serious problem.

Since synthesized models are rather difficult to handle and require quite large series of data, the authors proposed a more simple modelling approach, which is capable of examining the effects of global warming specifically. Predictive application of the model is favoured over ecological interpretation of mathematical procedures when modelling the seasonal dynamics within a simplified framework. When stressing few factors - often temperature only - we expect factors not included in the model to appear hidden or within the temperature term. Such models were fitted to the seasonal dynamics data series of aquatic organisms successfully. Considering temperature as the only factor determining seasonal dynamics, we are interested in whether temperature in itself may create similar pattern to the observed one. After fitting the model successfully to the data series of several years we get a plausible answer of it. On the basis of such models, populations of certain organisms are expected to peak earlier during the season, whereas the trend in changes of total abundance is varying. Furthermore, modelling the phytoplankton in the River Danube indicates large fluctuations between years due to the global warming. These models may support evidence of such future changes that we would not be able to forecast on the basis of our recent knowledge on the environmental changes.

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