Impact of *Conyza canadensis* on its Co-occurring Plant Species in its Non-native Region

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Abstract

The paper reports about the impact of *Conyza canadensis* on species that co-occurs with it in invaded and un-invaded plots at the various sites located in Kashmir Himalayas. These invaded and un-invaded plots supports diverse plant species varying in their status like native and non-native species which were at different stages of invasion, including invasive species, naturalised species, casual alien and cultivated un-escaped alien. Various ecological factors, such as disturbance and pollution have caused prominent changes in the dynamics and distribution of the native species of Kashmir Himalayas. The paper highlights the adverse impact of the invasive species on its co-occurring native species and facilitative role to non native aliens. Higher number and better performance of alien species in invaded than un-invaded plots in comparison to higher number and better performance of native species in un-invaded plots indicates invasion meltdown.

Keywords: Invasive species, naturalised species, casual alien, cultivated un-escaped alien, invasion meltdown

Introduction

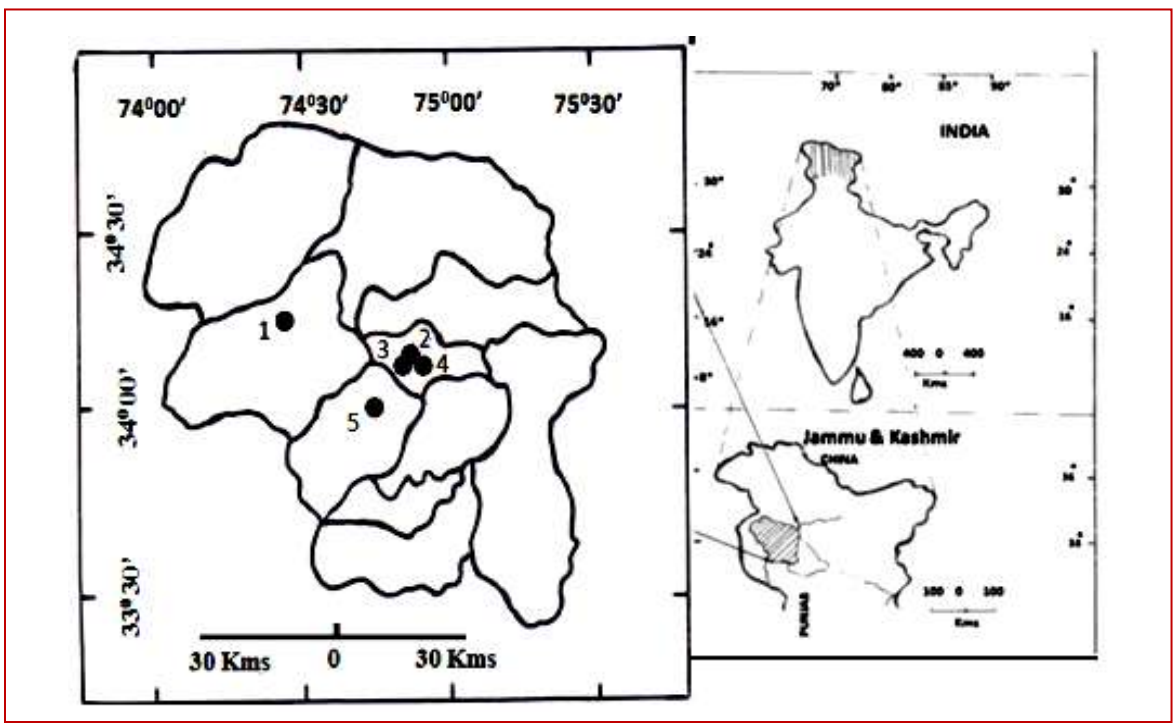
Plant invasion is one of the most severe threats to the biodiversity on Earth (Chapin *et al.*, 2000; Werner *et al.*, 2010) as exotic plant invaders appear to achieve disproportional dominance in their nonnative ranges through many mechanisms (Hierro *et al.*, 2005; Callaway *et al.*, 2008; Lankau *et al.*, 2009; Lankau, 2012). Various studies on these invasive species have reported that these species are rare at home and abundant or even superabundant in introduced communities (Bruce *et al.*, 1997; Paynter *et al.*, 1998; Memmot *et al.*, 2000), such observations remain largely anecdotal without any support based on quantitative data. Thus it is clear that those plants which are vulnerable to become superabundant only in the introduced range must be 'doing something different' in recipient communities that enables them to attain such dominance. Crawley (1987) was one of the first researchers who found that non native often were larger in size than their native conspecifics. Similarly Grosholz and Ruiz (2003) have observed the same pattern for marine invertebrates. Further, Jakobs *et al.* (2004) in his survey of 46 native and 45 introduced populations of *Solidago gigantea*, found that total plant biomass was larger among exotic than native plants. Callaway *et al.* (2011a) found that the abundance of *Acroptilon repens* in North America, where it is invasive, was almost twice that in Uzbekistan, where it is native. This difference in impact corresponded with inherently stronger competitive and allelopathic effects of *A. repens* on North American species than on species native to Uzbekistan (Ni *et al.*, 2010). Similar comparisons between native and nonnative ranges have been reported for the allelopathic effects of other invasives, including *Ageratina adenophora* (Inderjit *et al.*, 2011), *Centaurea stoebe* (Thorpe *et al.*, 2009), *Centaurea diffusa* (Callaway and Aschehoug, 2000), *Prosopis juliflora* (Kaur *et al.*, 2012), *Foeniculum vulgare* (Colvin and Gliessman, 2011), the red algae *Bonnemaisonia hamifera* (Svensson *et al.*, 2013), *Chromolaena odorata* (Qin *et al.*, 2013).

In this context, the present study was conducted to assess the impact of the native North American plant, *Conyza canadensis* (Asteraceae, *Erigeron canadensis*, commonly known as Canadian horseweed), on its co occurring plant species in Kashmir Himalayas; where it is highly invasive plant species. Studies of invasive species often focus on species that undergo dramatic increases in abundance in their nonnative ranges (Inderjit *et al.*, 2011; Kaur

et al., 2012). We tackled this issue through field studies at the various sites located in Kashmir Himalayas so as to evaluate the impact of *Conyza canadensis* on its neighbours that co-occur with it at the invaded plots with reference to un-invaded plots.

Material and Methods

In Kashmir Himalaya, India, five sites (Site 1: Khawajabagh-Baramulla; Site 2: Mirzabagh; Site 3: Kashmir University Botanical Garden; Site 4: Nigeen and Site 5: Garend-Berwah) (Figure 1) which were invaded by *Conyza canadensis* varying in the level of soil disturbance, spread over three districts were selected in Kashmir Himalaya representing diverse habitat types viz., terrestrial open habitat with low soil disturbance (LSDH), terrestrial open habitats with intermediate level of soil disturbance (ISDH), and riparian habitats. Within each site invaded and comparable un-invaded (control) plots were located. Criteria for site selection were that the survey should include range of habitats varying in the level of soil disturbance. For instance, the sites included terrestrial open habitats with low soil disturbance, terrestrial open habitat with intermediate level of soil disturbance and riparian habitat, (established several years ago and with a dense vegetation cover) (Table 1). The selected sites varied widely in the density or cover of *C. canadensis* and its co-occurring neighbours. At all the selected sites, *C. canadensis* stands formed distinct patches of one to several meters diameter within the vegetation. In this study, plots at the selected sites with either no or negligibly small number of individuals of *C. canadensis* were considered as "un-invaded or control plots", and stands with huge number of *C. canadensis* were called "invaded plots", although invaded plots also contained few native species. Plant species along with the *Conyza canadensis* were recorded quarterly during summer season.



1= Baramulla; 2= Mirzabagh; 3= Kashmir University; 4= Nigeen; 5= Budgam

Figure 1: Map of the study area showing distribution of sampling sites for different field studies in Kashmir Himalaya.

Table 1: Description of the sites in Kashmir selected for the study of *Conyza canadensis* and its co-occurring neighbours during the present study.

Sites	Site name/ Sampling location	District	Habitat type	Latitude N	Longitude E	Altitude m.a.s.l
S1	Khwajabagh	Baramulla	Open, dry, less disturbed	34°13′09"	72°22′42"	1598
S2	Mirzabagh	Srinagar	Dry, exposed, moderately disturbed or Intermediate	34° 07′46"	74° 49′54"	1590
S3	KUBG	Srinagar	Open, Dry, protected	34° 08′50"	74°50′ 11"	1580
S4	Nigeen	Srinagar	Open, Slightly Moist, highly disturbed	34°07′20"	74°50′00"	1580
S5	Garend-Beruwah	Budgam	Open, riparian, highly disturbed	34°03′07"	74°40′49"	1600

Results and Discussion

Field studies revealed that plant species growing in association with *Conyza canadensis* at the five selected sites of Kashmir Valley comprised 74 species belonging to 60 genera and 25 families (24 dicot and 2 monocot families). Family Asteraceae (18 species) and Poaceae (11 species) contribute maximum number of plant species distributed in 15 genera of Asteraceae and 10 genera of Poaceae (Table 2 and 3). Alien plant species belonging to Asteraceae were the worst alien species among all due to their fertility and unique seed structures which make them a very powerful colonizer in new environments especially in case of *C. canadensis*. Moreover, it was found that all of these *C. canadensis* invaded plots suffer from serious invasion. The surveyed plant species growing in association with *C. canadensis* in the Kashmir Valley included both native and non-native species which were at different stages of invasion, including 37 Invasive species (widespread and dominant), 14 naturalised species (established with self sustaining populations), 1 casual alien (occasional species with no self replacing populations), and 1 cultivated un-escaped alien (Figure 2). Besides, 21 native species of Kashmir Himalaya were recorded growing in association with *C. canadensis.* Most of these alien plants in the present checklist 31 were natives to Europe followed by Africa 16, (Asia 13, South America 5 and North America 3 respectively. Two probable reasons for such high number of European species in the alien flora of Kashmir Himalaya could be: (a) successful introduction due to more or less similar climate, and (b) European colonial past that could have facilitated the transport of plant propagules from Europe to this region with men and machinery.

Out of 74 species, the invaded and un-invaded plots harboured 58 and 62 species, respectively. In case of invaded plots out of 58, 47 plant species (1causal, 12 Naturalised, 34 invasive) were alien and only 11 were native while in case of un-invaded plots out of 62, 42 plant species (1 causal, 1cultivated, 8 naturalised, 32 invasive) were alien and only 20 were native. Therefore, an increase in the number of plant species was observed in case of un-invaded plots compared to invaded plots. The number of invasive species is higher in invaded plots compared to un-invaded plots (Figure 3). Our results are consistent with the results of with many other studies (Bimova *et at*., 2004; Dunbar and Facelli, 1999; Kohli *et al.*, 2004; Dogra *et al.*, 2009 a, b) which reported negative impact of exotic species on species diversity. In case of *Lantana camara*, Dobhal *et al.* (2011) also reported a decrease in species number and diversity in invaded localities as compared to un-invaded ones and thus such study is also in conformity with our results. Similar results have been obtained by Rascher *et al.* (2011) who reported decrease in species number and diversity by upto 50% in invaded compared to un-invaded areas of *Acacia longifolia.*

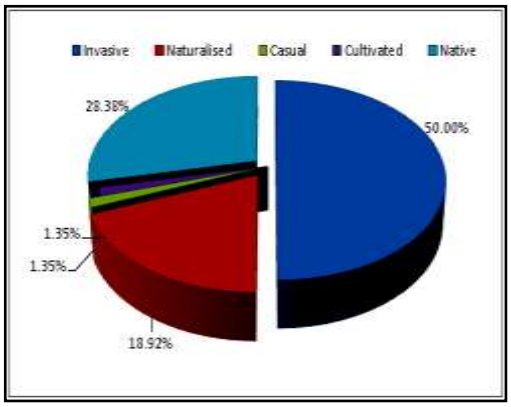


Figure 2: Distribution of the total plants species growing in association with *Conyza canadensis* in the invaded and un-invaded plots.

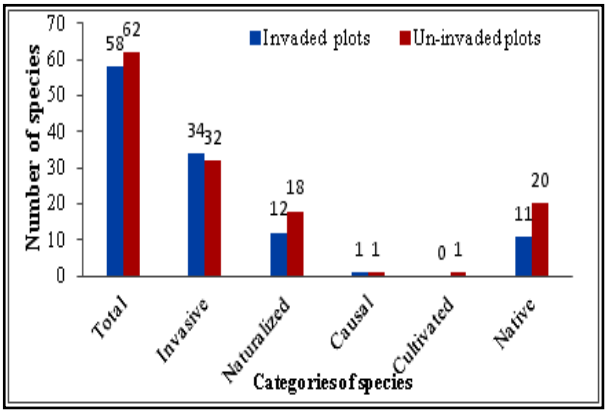


Figure 3: Impact of invasion by *Conyza canadensis* on the number of native and alien species in invaded and un-invaded plots.

Table 2: Conspectus of the alien plant species, families, origin, status, group, growth form and occurrence in plots invaded and un-invaded by *Conyza canadensis.*

Family/ plant species	Origin	Status	Group	Growth form	Mode/purpose of introduction	Occurrence	
						Inv	Un
Amaranthaceae							
Amaranthus caudatus L.	AMS	In	Dicot	A	Fd	+	+
Apiaceae							
Conium maculatum L.	EU	Nt	Dicot	B	Fd	+	+
Daucus carota L.	AF;EU	In	Dicot	B	Ui	+	+
Eryngium billardieri Del.	AF;EU	In	Dicot	P	Ui	+	+
Torilis japonica DC.	AS	Nt	Dicot	A	Ui	+	-
Asteraceae							
Achillea millefolium L.	EU	In	Dicot	P	Md	+	+
Anthemis cotula L.	EU	In	Dicot	B	Ui	+	+
Arctium lappa L.	EU	In	Dicot	P	Md	+	+
Artemisia absinthium L.	EU	In	Dicot	Ss	Md	+	+
Artemisia tournefortiana Reichb.	AS	Nt	Dicot	A	Ui	+	-
Cichorium intybus L.	EU	In	Dicot	P	Ui	-	+
Cirsium arvense Scop.	AS	In	Dicot	P	Ui	+	+
Galinsoga parviflora Cav.	AMS	In	Dicot	A	Ui	+	+
Taraxacum officinale F.H.Wigg.	AF	In	Dicot	P	Ui	+	+
Xanthium spinosum L.	AF	In	Dicot	A	Ui	-	+
Xanthium strumarium L.	AMN	In	Dicot	A	Ui	+	+
Brassicaceae							
Capsella bursa-pastoris Medic.	EU	In	Dicot	A	Ui	-	+
Sisymbrium loeselii L.	AF; EU	In	Dicot	A	Ui	+	+
Cannabiaceae							
Cannabis sativa L.	AS	In	Dicot	A	Ui	+	+
Chenopodiaceae							
Chenopodium album L.	EU	In	Dicot	A	Fd	+	+
Convolvulaceae							
Convolvulus arvensis L.	EU	In	Dicot	P	Ui	+	+
Cyperaceae							
Cyperus globosus All.	AF; EU	In	Monocot	A	Ui	+	-

Family/ plant species	Origin	Status	Group	Growth form	Mode/purpose of introduction	Occurrence	
						Inv	Un
Euphorbiaceae							
Euphorbia helioscopia L.	AS; EU	In	Dicot	A	Ui	+	-
Fabaceae							
Medicago polymorpha L.	AF; EU	In	Dicot	A	Fr	+	+
Medicago sativa L.	AF; EU	Nt	Dicot	B	Fr	+	+
Robinia pseudoacacia L.	AMN	In	Dicot	T	Pl	+	-
Trifolium pratense L.	EU	In	Dicot	P	Fr	+	+
Trifolium repens L.	EU	In	Dicot	P	Fr	+	+
Lamiaceae							
Marrubium vulgare L.	AS; EU	In	Dicot	P	Ui	+	-
Mentha longifolia L.	AF; EU	In	Dicot	P	Ui	+	+
Nepeta cataria L.	EU	Nt	Dicot	P	Ui	+	+
Malvaceae							
Althaea rosea Cav.	AS	Cs	Dicot	B	O	+	+
Onagraceae							
Oenothera rosea Ait.	AMS	In	Dicot	A	Ui	+	+
Plantaginaceae							
Plantago lanceolata L.	AF; EU	In	Dicot	P	Ui	+	+
Plantago major L.	EU	In	Dicot	P	Ui	+	+
Poaceae							
Boyhriochloa ischaemum Keng.	AF	In	Monocot	P	Ui	+	+
Bromus japonicas Thunb.	EU	Nt	Monocot	A	Fr	+	+
Echinochloa crus-galli Beauv.	AS	Nt	Monocot	Aq	Ui	+	-
Lolium perenne L.	AS; EU	Nt	Monocot	P	Fr	-	+
Oryza sativa L.	AS	Cl	Monocot	A	Fd	-	+
Phragmites australis Trin.	AMS	In	Monocot	P	Fr	+	-
Setaria viridis P. Beauv.	AS; AF	In	Monocot	A	Fr	+	+
Sorghum halepense Pers.	EU	In	Monocot	P	Fr	+	+
Sorghum vulgare Pers.	AF	Nt	Monocot	A	Fr	+	-
Polygonaceae							
Polygonum hydropiper L.	EU	In	Dicot	A	Fd	+	+
Rumex dentatus L.	AF; EU	Nt	Dicot	A	Md	+	+
Portulacaceae							
Portulaca oleracea L.	AF;	Nt	Dicot	A	Fd	+	+
Rosaceae							
Fragaria nubicola Lindel. ex.	EU	Nt	Dicot	P	Fd	+	-
Potentilla reptans L.	AS; EU	Nt	Dicot	P	Ui	-	+
Rubus ulmifolius Schott.	EU	In	Dicot	S	Ld	+	+
Rubiaceae							
Rubia cordifolia L.	AS; AF	Nt	Dicot	C	Ui	+	-
Solanaceae							
Datura stramonium L.	AMN	In	Dicot	A	In	+	+
Urticaceae							
Urtica dioica L.	AF; EU	In	Dicot	P	Ui	+	+

Abbreviations:

Origin: AMN = North America; AMS = South America; EU = Europe; AF = Africa; AU = Australia; AS = Asia (excluding the Indian sub-continent);

Growth form: A = Annual herb; B = Biennial herb; P = Perennial herb; Ss = Sub shrub; S = Shrub; T = Tree; Aq = Aquatics; C = Climber

Mode of introduction: Fd = Food; Fr = Fodder; Ld = Landscaping; Md = Medicinal; O = Ornamental; Ui = Unintentional Invasion status: Cl= Cultivated un-escaped aliens; Cs = Casual aliens; Cn = Casual or naturalized aliens; Nt = Naturalized aliens; In = Invasive alien, Inv= invaded plots, Unv = uninvaded plots

Table 3: Conspectus of the family wise native plant species, growth form, group and occurrence in plots invaded and un-invaded by *Conyza canadensis.*

Family/ plant species	Growth form	Group	Occurrence	
			Inv	Un
Asteraceae				
Artemisia dubia Wall. ex besser.	P	Dicot	-	+
Carpesium cernuum L.	P	Dicot	-	+
Cotula anthemoides L.	A	Dicot	-	+
Myriactis nepalensis Less.	A	Dicot	-	+
Lactuca serriola L.	B	Dicot	+	+
Leucanthemum vulgare Lam.	P	Dicot	+	+
Tragopogon kashmirianus G.S.	B	Dicot	+	+
Boraginaceae				
Cyanoglossum glochidiatum Wall. ex Benth.	A	Dicot	+	+
Myosotis arvensis L.	P	Dicot	+	+
Fabaceae				
Melilotus albus Medik.	A	Dicot	+	+
Geraniaceae				
Geranium nepalense Sweet.	A	Dicot	-	+
Malvaceae				
Malva sylvestris L.	A	Dicot	+	+
Apiaceae				
Eryngium caeruleum M. Bieb.	P	Dicot	+	+
Poaceae				
Cynodon dactylon L.	P	Monocot	+	+
Hordeum murinum L.	A	Monocot	-	+
Polygonaceae				
Polygonum lapathifolium L.	A	Dicot	-	+
Polygonum plebejum R.Br.	A	Dicot	-	+
Rosaceae				
Potentilla arvensis L.	P	Dicot	+	-
Potentilla nepalensis Hook.	P	Dicot	-	+
Scrophulariaceae				
Veronica agrestis L.	A	Dicot	-	+
Solanaceae				
Solanum nigrum L.	A	Dicot	+	+

Growth form: A = Annual herb; B = Biennial herb; P = Perennial herb.

In the plots invaded by *Conyza canadensis* the highest percentage of aliens were found to be in invaded plots of every study site and minimum in un-invaded plots. Amongst the natives, the highest percentage was observed in the un-invaded plots of each study site as compared to invaded plots. Reason for such an increase of alien species is due to "invasion meltdown" a process by which a group of non-indigenous species facilitate one another's invasion in various ways thereby increase the likelihood of survival and/or of ecological impact and possibly the magnitude of impact (Simberloff and Von Holle, 1999). Some of the plant species which have been found to facilitate invasion by other alien species are *Myrica faya* in Hawaii, where it has altered soil nutrients by invading very nitrogen poor volcanic soils (Vitousek and Walker, 1989). *Mesembryanthemum crystallinum,* African Crystalline ice plant, that modifies the environment to favour other introduced species in California (Philbrick, 1972; Vivrette and Muller, 1977) and Australia (Kloot, 1983). Highly disturbed habitats which provide "windows of opportunity" for the entrance of alien propagules (Myster, 1993) may increase the quantity of available space for establishment and growth of alien species (Davis *et al.*, 2000), other likely reasons for having higher number of aliens as compared to number of natives in invaded plots. Altered competitive interactions between the species caused by anthropogenic activities operating at small scale create

new niche opportunities for recruitment and establishment of species and as a result of such altered competitive interactions might have reduced biotic resistance and facilitated the establishment of exotic species (Verdu and Valiente-Banuet, 2008; Altieri *et al.*, 2010).

Moreover, the findings of our study also revealed higher number of invasive species in plots invaded by *C. canadensis.* It could be due to soil modification done by invasive species by employing various ways that facilitate invasion by other species as well. It is well known fact that invasive species alter physical chemical attributes of soil including cycling of nutrients and other elements (Haubensak *et al.*, 2004; Hawkes *et at.*, 2005), pH (Kourtev *et al.*, 2003), soil organic matter and soil particle aggregation (Saggar *et al.*, 1999). Other findings have reported more direct effect on the biotic composition of invaded soil, e.g., alteration of soil food web (Duda *et al.*, 2003). Total soil microbial communities (Kourtev *et al.*, 2003) mutualistic fungi (Mummey and Rilling, 2006; Jordan *et al.*, 2008) are also reported to be altered by invasive species which in turn facilitates invasion directly or via cross facilitation of other invasive species. Our results are also consistent with McIntyre *et al.* (1988); McIntyre and Lavorel (1994a); Hoffmann (1998), who also revealed that high species richness of alien plants is coupled with low species richness of native plants in man-dominated habitats and with high richness in natural habitats.

Higher percentage of other co-occurring non-natives /aliens in the plots invaded by *Conyza canadensis* than native plant species indicates potential role of *C. canadensis* in the success and spread of its co-occurring aliens in the disturbed habitats of the Kashmir Himalaya (Figure 4). Our results are consistent with the results of many other studies (di Castri, 1989; Kornas, 1990; McIntyre and Lavorel, 1994b; FaliNski, 1998; Pysek, 1998; Sukopp, 1998; McKinney, 2002) who also reported that ecosystems with anthropogenic disturbances, contain high numbers of aliens, whereas natural or near-natural ecosystems display a certain ecological resistance against the introduction of alien species (FaliNski, 1998). McIntyre and Lavorel, (1994b); Hoffmann (1998), have revealed that high species richness of alien plants is coupled with low species richness of native plants in man-dominated habitats and with high richness in natural habitats. Likewise low community resistance against invading non-native species is caused due to less number of native plant species thereby indicating that native plant species increases community resistance against invading non-native species (Levine, 2000), at least as long as disturbance levels are low (Cornell and Karlson, 1997).

These results add to the growing body of literature reporting *Conyza canadensis* does have a negative relationship with native species richness in its non native range, and it significantly decreases native species richness in its non-native (Kashmir Himalaya). It is pertinent to mention that Shah *et al.* (2014) recently demonstrated through transcontinental field studies, green house experiments and individual based models that *C. canadensis* significantly reduces the native plant diversity in non-native ranges but not at home.

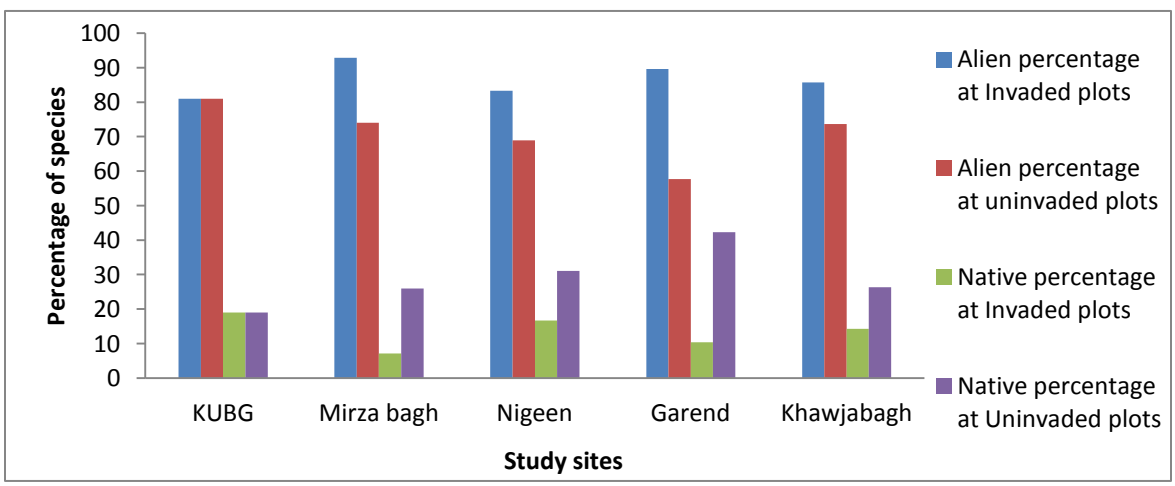


Figure 4: Percentage comparison of co-occurring aliens and native plant species in plots invaded and un-invaded by *Conyza canadensis.*

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Wood Anatomical Features of *Juglans regia* L. from Temperate Climate of Kashmir Himalaya

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Abstract
Walnut wood an important natural resource is used in high class and decorative joinery for furniture and tableware, and also in decorative construction work such as in Mosques, houses, *Khanqaas*, Shrines and other public and private buildings, to which it is eminently suitable due to its strength and durability at the same time relatively little is known about anatomical characteristics of its wood. For this purpose sections were cut in three different planes and also maceration was done in order to know about wood anatomical features viz. fiber length, fiber diameter, fiber wall thickness, vessel element length and vessel element diameter, growth ring.
Keywords: *Juglans regia*, walnut, fiber, wood, anatomy.

Introduction

Walnuts are large, monoecious trees with wide, dense crowns that can reach heights of 15–35 m. Older trees may have trunk diameters upwards of 1.5–2.5 m (Shalit, 1951). Young trees have slightly furrowed, light-grey bark, while older trees have bark that is darker-grey and strongly furrowed. The leaves are alternate, 19–54 cm long and 15–40 cm wide, imparipinnate with 3–5 pairs of leaflets. The leaflets are typically dark-green, ovate, coriaceous and glabrous, with entire margins. The male flowers are arranged in catkins, with each flower comprising 8–40 stamens. The female flowers occur in groups of 1–3 on the ends of young branches. The fruit is drupe-like and spherical, with a green, dehiscent pericarp (husk), which releases the nut when mature. Theendocarp, or "shell", is light brown and hard. The kernel is covered with a thin, yellow to brown papery layer (pellicle). Western Himalayan especially valley of Kashmir produces high quality walnuts. The Jammu and Kashmir State alone accounts for >98% of India's total production with an average productivity of 2.69 metric tonnes/ha from an area of 83613.80 ha and production of 224595.85 metric tonnes (Sharma, 2012). India export around 5000 metric tonnes walnut kernel of worth US $ 260-300 million annually to France, Germany, Spain, Portugal, Austria, United Kingdom, Kuwait, Bahrain, Dubai and Saudi Arabia. Besides, domestic market of worth of US $ 140-200 million for kernel and in shelled walnuts is also fulfilled by the state (Per. Com. with J and K Walnut Exporters Association in 2012). Also the wood of walnut is hard and strong, stable, lightweight, shock-resistant, flexible. It shrinks and swells less than almost any other wood. It's sweet to work, lovely to smell, delightful to handle, and takes a splendid finish.' (McIntosh, 1995) The wood is mostly used in high class and decorative joinery for furniture and tableware and also for Mosques, Shrines, *Khanqaas*, and other public and private buildings. Wood anatomy an important branch of wood science; which is suitable for predicting the varied utility of woods for different purposes, the present work is an attempt to elucidate wood anatomical patterns in *Juglans*, from temperate climate of Kashmir Himalaya.

Material and Methods

Source of Material: The present study was carried out on hardwood trees of *Juglans regia* L. with deciduous type of habit, belonging to the family *Juglandaceae* from natural provenances of Kashmir Himalaya for their anatomical characteristics.

Samples of approximately 1 cu cm in size were taken at breast height (1.3 m) and were softened by boiling in water for 10-15 minutes. Cross, radial and tangential sections of 15–20 µm thick were prepared by using Reichert microtome, as well as sharp razor for studying various wood anatomical microscopial features. These features were identified as per International Association of Wood Anatomists (IAWA) list of microscopic features for hardwood identification (Wheeler *et al.,* 1989).

Maceration: Maceration was done as per Jeffery's method (Johansen, 1940) for all the sample studies. In this method, small slivers of wood were taken from the samples collected and put into the test tube and then filled with 10% chromium trioxide and 10% nitric acid and left for one to several days at room temperature and the process was hastened by heating up to approximately 60^0C for few minutes. After that, the material was thoroughly washed with distilled water till traces of the acid were removed. The mixture was teased/shaken thoroughly to separate the wood elements and stained with 1% Safranin and mounted in glycerine on microscopic slides.

Staining Procedure for Sections: Twenty micron thick; transverse, radial and tangential sections were stained in Heidenhains haematoxylin and safranin for 20 minutes. The stained sections were washed in acetone and xylene of 1:1 ratio for 10 minutes to ensure complete dehydration and subsequently in Xylene. Finally they were mounted in Diphenyl Pthalate Xylene (DPX) mountant to make the permanent slides.

Photomicrography: Photomicrography involves combination of the principles of microscopy and photography; is a technique of recording microscopic image. By this method, the object was focussed under microscope and the photographs were taken with the help of Olympus Clinical CH20I microscope model CH20 BIMF 200; on the top of which Olympus camera was fitted with photomicrographic attachements.

Wood Element Measurements: The measurements/dimensions of different wood elements viz., vessel elements and rays were made from macerated and transverse section materials, with the help of ocular-stage micrometry. Twenty-five measurements were made from unbroken fibers and vessel elements for lumen diameter, wall thickness, length and width.

Colour: The colour feature was determined when logs were cut across, showing two distinct regions of sapwood and heartwood (Chowdhury and Ghosh, 1958) and (Rao and Junjea, 1971).

Some timbers when cut on the surface have some characteristic smell or odour due to resins, oils or chemical deposits. It may be asset or a liability in its utilization for a given purpose. This feature was determined by exposing fresh wood sample or by adding moisture through breathing already cut wood sample (Wheeler *et al.,* 1989).

Results and Discussion

Juglans regia Linn.

Sapwood greyish-white, broad; heartwood greyish-brown with few or no markings or with darker streaks; the wood varies considerably in intensity of colour and in markings, and beautifully mottled stock can be obtained by selection; rather lustrous, working to smooth surface under tools, without odour or taste, straight grained, medium- and quite even textured.

Wood diffuses porous with medium to large pores. Growth rings distinct, delineated by 1-3 layers of flattened fiber tracheids and one layer of wood parenchyma, narrow to little, 0.3-2.3 mm. Pores few arranged in radial direction, 5-8/mm sq, medium to large, gradually decreasing in size towards the end of the growth rings, tending to be semi-ring porous mostly solitary or in small radial multiples of 2-4. Solitary pores oval or round in outline 50-250 x 50-299 µm, thin walled, 2.5 µm. Vessel element 170-900 µm long; perforation plates exclusively simple; intervessel pits dense, compact and alternate, a little large polygonal in outline, 5-12.5 µm in diameter, with slit like apertures. Helical thickenings not observed. Tyloses abundantly present. Nonperforated tracheal elements

fiber tracheids, consisting ground mass of the wood, square, polygonal or oval in cross section, 10-40 µm in diameter, a little thick walled, 2.5 µm; bordered pits round, 5 µm in diameter. Wood parenchyma apotracheal, diffuse aggregate in narrow discontinuous bands, similar to fiber tracheids in cross section, thin walled, 1.2 µm, crystals not found. Rays homogenous, uniseriate and multiseriate. Uniseriate rays low, 2-11 cells or 50-350 µm tall. Multiseriate rays wide and rather low, 2-7 cells or 20- 81 µm wide, and 210-900 um tall with uniseriate wings of 1-11 cells tall. Procumbent cells small oval or vertically elliptical in tangential section, 15-28, 10-20 x 55 -120 µm. Ray vessel pits dense, alternate, oval a little large, 5-12 µm in diameter (Figure 1, 2, 3 & 4).

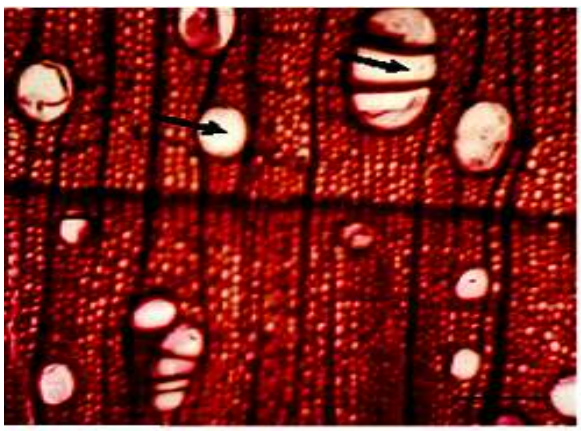

Figure 1

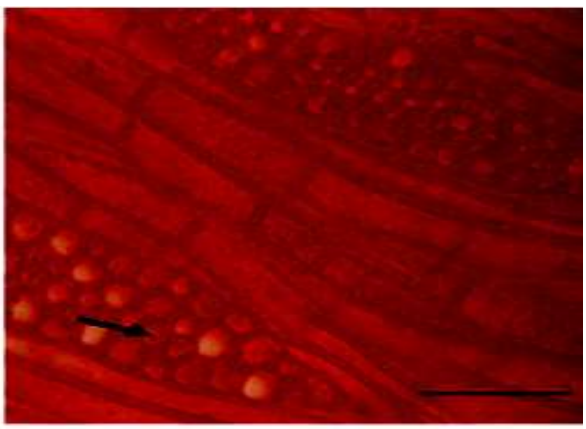

Figure 2

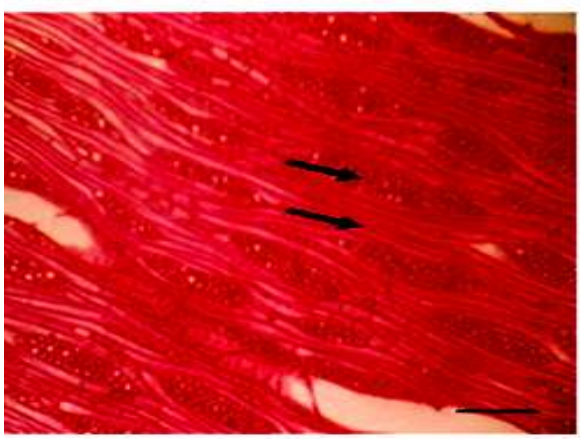

Figure 3

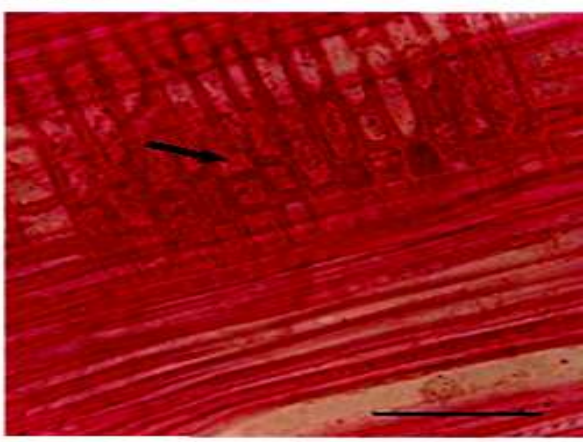

Figure 4

Figure 1: Transverse section showing pores mostly solitary or in radial multiples of 2-4; oval or round in shape; **Figure 2 and 3.** Transverse longitudinal section showing broad multiseriate rays, intermingled with fibers; **Figure 4.** Radial longitudinal section showing homogeneous rays (Scale bar Figure 1 and 3 = 50 µm, Figure 2 and 4 = 40 µm).

Wood anatomical structure of *Juglans regia* linn. growing in the Kashmir Valley was investigated. According to the records in the literature, there have not been any studies on the wood anatomy of walnuts in Kashmir except few studies by Wani and Khan (2008, 2010 and 2013). Further these studies were mainly oriented towards the wood variability and cambial activity in *Juglans regia*. The most detailed information on wood anatomy of *Juglans regia* is given by Metcalfe and Chalk (1965) in *Anatomy of Dicotyledons.* Also Safdari *et al.* (2008) provided hand lens wood anatomy of walnut with schematic and anatomical illustrations. In the present study, the Sapwood of *Juglans regia* was found greyish-white broad and heart wood greyish brown with few or no markings

or with darker streaks .Same was reported by Timar *et al*.(2010) The sap wood of a new wood provides a pipeline for the movement of water and nutrients through the trunk and into the leaves (Medhurst and Beadle, 2002). The heartwood was found uniformly light red at first and afterwards turns light reddish brown with passage of time and having irregular contour in the transverse section. Also in the present study, wood of *Juglans regia* was found to be diffuse porous with distinct growth rings. Vessels were few mostly in short to long radial multiples of 2-4. Vessel elements were found to be long with simple perforations and with alternate intervessel pits. Libriform fibres were thin walled ,long with simple pits mainly restricted to radial walls . Axial parenchyma was narrow and with discontinous bands. Rays uniseriate as well as multiseriate. The similar results were obtained by Metcalfe, (1939); Miller,(1976); Blokhina, (2007); Safdari, *et a*l (2008) and Timar *et al* (2010, 2013)

Conclusions

Wood anatomical studies of the *Juglans regia* Linn**.** a versatile natural resource of Kashmir Himalaya which is chiefly used in high class and decorative joinery for furniture and tableware, have been carried out . The dimensions of cellular structures viz., vessels, fibers, rays, and parenchyma present in the *Juglans regia*, which have direct bearing on the properties of wood, have been discussed.

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Consequences of Lake Deweeding on Periphytic Algae: A Case Study of Dal Lake, Kashmir

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Abstract
Environmental effects of harvesting have been reported in aquatic ecosystems around the globe. An ecological monitoring study was undertaken to ascertain the impacts of this lake management practice on the structure and distribution of Periphyton community of Dal lake ecosystem. Deweeding significantly reduced periphytic diversity and density ($P<0.05$). Upon initiation of deweeding process, maximum number of dominant taxa got removed along with the harvested weeds, which as a result provided an opportunity for few rare taxa to thrive at such sites. Bacillariophyceae was the dominant group in all seasons. Cyanophyceae (*Oscillatoria* sp. and *Anabaena* sp.) registered slight rise after deweeding. The species of *Achnanthidium, Synedra, Chlorella, Navicula, Cymbella and Fragillaria* were observed to be highly prominent at deweeding sites. Due to harvesting operation, species composition and distributional pattern of this very important autotrophic community got altered. The present study concludes that there was a negative impact on periphyton community by deweeding process soon after implementation of this practice. The recovery rate of studied periphytic community was slow. In order to ascertain the full recovery of periphytic community, long term ecological monitoring (>10 years) is mandatory.
Key words: Deweeding, periphyton, lake ecosystem

Introduction

Biological communities have been shown to be useful indicators of general water quality (Belmont and Counties, 2015; Martinez-Haro *et al.*, 2015). Biological monitoring, or biomonitoring, is therefore used as an integrator of various stressors that provides valuable information about the overall integrity of a water body. While chemical monitoring is conducted to support established water quality criteria and some priority pollutants, chemical monitoring alone cannot ensure that all pollutants and interactions among them are meeting water quality goals. Biological organisms serve not only as useful indicators of current conditions, but also of cumulative effects and changes over time (Morin *et al.*, 2015).

Periphyton is an important component of aquatic ecosystems, providing food for invertebrates, and also acts as a bio-indicator (Finlay *et al.*, 2002). Excessive periphyton growth can occur in aquatic systems as a result of nutrient enrichment and entry of effluents from wastewater treatment facilities. Therefore the occurrence and abundance of the group can serve as an indicator of the health of the concerned aquatic system (Cascallar *et al.*, 2003; Giorgi and Malacalza, 2002). The assemblage also plays a very important role in aquatic food webs by providing a readily available energy source for a wide range of aquatic organisms. Periphytic algae reproduce and respond rapidly to environmental change and provide early warning indicators of both pollution increases and habitat restoration success (Stevenson and Pan, 1999).

Periphyton is sensitive to changes in water quality and, in particular, responds rapidly and predictably to nutrient enrichment in lakes and streams (Horner *et al.* 1983; Stevenson *et al.* 1985; Cattaneo, 1987; Biggs, 1988; Lowe,

1996). The short generation times of algae allow them to respond more rapidly to changes in water quality than macrophytes or fauna. Certain structural (i.e. species shifts) and functional i.e. growth rates changes in the Periphyton are symptomatic of nutrient enrichment and provide an early signal of eutrophication. Reference conditions can be determined by characterizing Periphyton in least affected areas.

Lake restorations are usually attempted to improve water quality or to improve aesthetic and recreational needs (Gangstad, 1982). Macrophyte harvest (Deweeding) differs from other restoration approaches in that these techniques involve physical removal of material from the water body. The idea behind deweeding as a restoration technique is that it will reduce the internal productivity of the water body and remove phosphorus that is stored in the plant by removing the plant. Harvesting is also done for social and recreational reasons; most people think of all aquatic vegetation as weeds and do not want the vegetation in their lake. All harvesters are generally based on designs by Wisconsin researchers (Cooke *et al.,* 1986). The units are paddle wheel propelled and have a large frame extending down into the water ahead of the bow (Figure 1). This frame is made up of vertical sickles on the sides and a sickle across the bottom connecting the two sides. A conveyer belt extends up from this frame to the boat and carries the cut macrophytes to the surface where they are collected (Cooke *et al.* 1986). The main advantage of macrophyte harvest is that it is a highly visible technique that provides instant results. The main disadvantage is that the process does not remove a significant amount of phosphorus from the system.

As ecosystem is a delicate web of organisms, the above restoration activity was thought to impact the entire biological community and Periphyton is no exception. In order to have an insight into the impact of deweeding in the Dal Lake on the occurrence, abundance and distribution pattern of the Periphyton community, the present study was undertaken.

Figure 1: Mechanical harvesters (Aquarius systems) in Dal Lake.

Material and Methods

Sampling schedule

For the present study, sampling was carried out on bi-monthly basis for a period of two consecutive years to evaluate the impact of ongoing deweeding on the community structure and distributional pattern of Periphyton of Dal Lake.

Study sites

For the present study, fourteen sites (Figure 2 and Table 1) were taken across the length and breadth of Dal Lake to assess the composition and distribution of Periphyton.

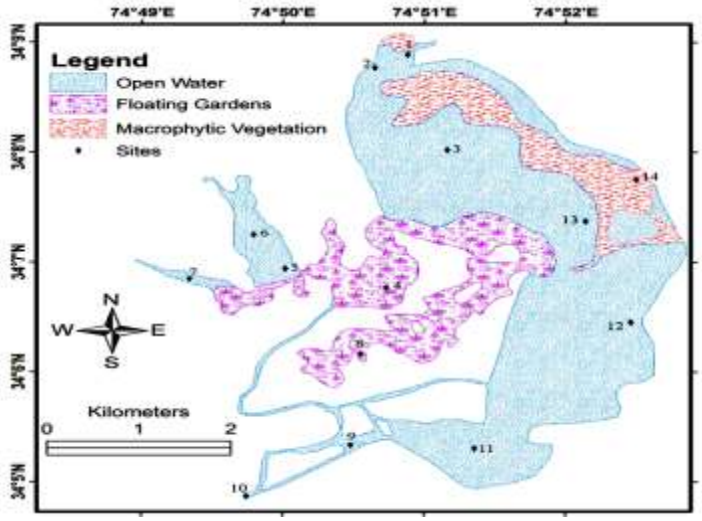

Figure 2: Map showing the Study sites

Table 1: Location of the sampling sites

Site	Name of Site	Location	Site	Name of Site	Location
1	Inflow - I	Telbal Nala	8	Floating Garden –II	Kandmohalla
2	Inflow -II	Boutkul Near Habak STP	9	House Boat -II	Bouleward road near
3	Hazratbal open	Between Hazratbal	10	Dal exit -II	Near Dal lock gate
4	Floating	Near Ashai Bagh	11	Gagribal open	Gagribal Basin
5	House Boat -I	Nigeen Basin	12	Deweeding –I	Between Centure hotel
6	Nigeen open	Nigeen Basin	13	Nishat open	Nishat basin
7	Dal exit -I	Pokhribal (Nigeen Basin)	14	Deweeding –II	Near Dockyard

Sample collection

For the collection of Periphyton, sampling was carried out on bi-monthly basis. The dominant macrophytes (natural substrates) were recorded at each site (Table 2). The aquatic plants collected for Periphyton included *Potamogeton natans, Trapa natans, Nymphoides peltatum, Potamogeton leucens, Ceratophyllum demersum, Myriophyllum spicatum and Hydrilla verticellata*. The plant leaves were packed in polybags and transferred to laboratory for further processing (Cattaneo and Kalff, 1978).

Sample processing

In the laboratory, each macrophyte was placed in a tray. The plant leaves were scraped with a sharp blade and then washed with distilled/deionized water (Cattaneo, 1978). Further, plastic bags were also rinsed with deionized water to procure detached algae. Host plant material if present, was removed from the epiphyte slurry. Epiphytic slurry after raised to a definite volume with distilled water was quantitatively subsampled for abundance and taxonomic composition (Gough and Woelkerling, 1976).

Preservation

Lugol's Iodine (Rice *et al*., 2005) and 4% formaline (Wetzel and Likens, 2004) was used for the preservation of Periphytic samples.

Table 2: Dominant macrophytes collected at each site

Sites	Dominant Macrophytes	Sites	Dominant Macrophytes
1	*Potamogeton leucens, Hydrilla verticellata*	**8**	*Ceratophyllum demersum, Nymphoides peltatum*
2	*Potamogeton crispus, Ceratophyllum demersum,*	**9**	*Ceratophyllum demersum, Hydrilla verticellata.*
3	*Potamogeton natans, Ceratophyllum demersum,Potamogeton crispus.*	**10**	*Hydrilla verticellata, Potamogeton leucens.*
4	*Nymphoides peltatum,Nymphaea mexicana.*	**11**	*Potamogeton leucens, Myriophyllum spicatum.*
5	*Ceratophyllum demersum, Potamogeton leucens,*	**12**	*Potamogeton natans, Potamogeton crispus.*
6	*Ceratophyllum demersum, Nymphoides peltatum*	**13**	*Ceratophyllum demersum, Potamogeton natans, Hydrilla verticellata, Potamogeton leucens.*
7	*Trapa natans,Hydrilla verticellata, Ceratophyllum demersum*	**14**	*Nymphoides peltatum, Potamogeton natans.*

Counting

Enumeration of Periphyton was carried either via strip or field counting by taking one ml of sub-sample in Sedgwick-rafter cell (Rice *et al*., 2005). In case, the material in Sedgwick-Rafter cell was found too dense to count directly, dilution technique was trailed. The morphology of many macrophytes (*C. demersum, M. spicatum, H. verticellata*) is of such a complex nature that determination of average surface area was difficult to determine, so individuals per10mg dry weight of macrophyte was followed in accordance with Zutshi and Ticku (1990).

Identification

For identification, standard works of Edmondson (1959), Prescott (1964), Adoni *et al.* (1985), Cox (1996), Biggs and Kilroy (2000) were followed. Periphytic algae were identified at 400X under Olympus binocular microscope. SEM technique was also employed for the identification and characterization of some of the species.

Sorenson's similarity coefficient (syn. coefficient of community, CC)

Uses presence/absence data:

$\mathbf{S_S} = 2a/ (2a + b + c)$,

Where, S_S = Sorensen similarity coefficient

a = number of species common to both Sites

b = number of species unique to the one site, and

c = number of species unique to the second site

S_S usually is multiplied by 100% and may be represented in terms of dissimilarity (i.e., $D_S = 1.0 - S_S$)

Results and Discussion

Myriophyllum and *Ceratophyllum* supported maximum no. of taxa while the minimum no. was recorded on Trapa. A total of 137 species belonging to 75 genera of periphytic algae were identified. Bacillariophyceae (88) was the dominant group found on all the sites followed by Chlorophyceae (41), Cyanophyceae (08) and Xanthophyceae (01).

Among the Bacillariophyceae, the dominant taxa were *Cymbella*, *Amphora, Navicula, Synedra, Cocconeis, Gomphonema, Fragillaria, Epithemia* and *Stauroneis*. The less dominant taxa included *Meriodon, Achnanthes, Achnanthidium, Nitzschia, Mougeotia, Craticula, Pinnularia, Cyclotella, Diploneis, Neidium, Asterionella, Selanastrum, Staurastrum* and *Tetraedon*. In Chlorophyceae, the dominant taxa include *Cosmarium*, *Microspora*,

Spirogyra, Lyngbya and *Scenedesmus.* Cyanophyceae were represented by *Oscillatoria* and *Anabaena.* Xanthophyceae was represented by *Vaucheria.*

On the basis of their morphological features the following categories of periphyton were distinguished:

i) Filamentous green algae (*Spirogyra, Ulothrix*)
ii) Prostrate or heterotrichous green alage (*stigeoclonium*).
iii) Unicellular algae, mostly diatoms, attached by mucilage (*Cymbella, Amphora*);
iv) Unicellular stalked(regular stalk or gelatinous stalk) algae(*Gomphonema*);
v) Unicellular forms loosely attached with or without mucilage (*Navicula*); and
vi) Small colonial algae loosely or firmly attached (*Scenedesmus*).

The Dal Lake is for most part infested with macrophytes of all the four recognized categories, viz., submerged, rooted free floating leaf, emergent and free floating types. A total of 31 species of macrophytes have been reported from the lake (Qadri and Yousuf, 2008). The submerged macrophytes like *Ceratophylum demersum, Myriophyllum spicatum, Hydrilla verticillata* and *Potamogeton crispus* are the most prominent ones that are removed during mechanical deweeding. These submerged macrophytes with their characteristic finely dissected and densely packed leaves provided an adequate shelter and food source for Periphyton (Bogut *et al.*, 2009).

Generally high number of Periphyton were found attached to *Myriophyllum spicatum and C. demersum* followed by *H. verticillata, P. crispus, P. natans* and least for *T. natans and N. peltata*. Periphyton growth on natural substrates was maximum in late autumn (Nov-Dec.) to early spring and minimum in summer. Overall, the highest population density (230×10^3 units/10mg.dw) was recorded at site 3(undisturbed) in late autumn. On the other hand, lowest density (10×10^3 units/10mg.dw) was observed at site 14(deweeding) during harvesting operation in spring.

Majority of predominant Periphyton occurred frequently at all the study sites but a few were site-specific. For instance, *Merismopedia* sp. was confined to deweeding sites. The Bacillariophyceae (diatoms) was much more diverse than Chlorophyceae (green algae) and Cyanophyceae (blue green algae) in the lake and in terms of mean density of individuals the group was also showing maximum occurrence.

Periphyton dynamics at undisturbed sites

Four Sites were selected which were undisturbed ones across the length and breadth of the Dal Lake. Each undisturbed site was selected from each basin of the Lake. These included Site5 (Hazratbal basin), Site9 (Nigeen basin), Site14 (Gagribal basin), and Site16 (Nishat basin). All these undisturbed sites showed a similar trend in terms of population density with increasing trend towards the late autumn and decreasing trends towards spring and summer. Overall high population density of periphyton was observed at Site 3 among various undisturbed sites.

Periphyton dynamics at deweeding sites

The process of deweeding in the Lake ecosystem took place twice every year in spring and autumn. These sites showed comparatively less number of Periphyton species, particularly during the deweeding operation, with slight increase afterwards. Bacillariophyceae was the dominant group in all seasons. Cyanophyceae (*Oscillatoria* sp. and *Anabaena* sp.) registered slight rise after deweeding. The species of *Achnanthidium, Synedra, Chlorella, Navicula, Cymbella and Fragillaria* were observed to be highly prominent at deweeding sites. Some of the sensitive species were not found in harvesting period primarily because of the habitat disruption due to deweeding.

During deweeding in summers the harvester prompted vigorous turbulence in the water column, which led to perturbation in the area in terms of suspension of sediments as it was revealed by the decrease in Sechi transparency. Habitat disturbance in the sites due to removal of plant biomass along with large-scale turbulence of water column was found to result in dislodging and removal of Periphytic algae. The removal of Bacillariophyceae

from the macrophytes was highest as compared to Chlorophyceae and Cyanophyaceae due to their numerical abundance.

The data revealed that the dominance of some species slightly decreased leading to uniformity of Periphytic algae. The plausible reason might be that prior to deweeding the community was composed of a few dominant and several less common as well as some rare taxa. Once there was deweeding, more number of dominant taxa got removed along with the harvested weeds, while the taxa that were rare before deweeding got better opportunities to increase in their population. The list of the Periphytic species which were exclusively present in Dal Lake is given in Table 3.

Table 3: Distributional pattern of periphytic algae among undisturbed and deweeding sites in the Dal lake ecosystem

Taxa	Undisturbed Sites	Deweeding Sites
Bacillariophyceae		
Achnanthes affinis	+	+
Achnanthes clevei	+	-
Achnanthes pseudoswazi	+	-
Achnanthes rostrata	+	-
Achnanthes hungarica	+	+
Acnanthidium delicatulum	+	-
Achnanthidium lanceolatum	+	-
Achnanthidium minutissima	*A*	*A*
Achnanthidium rosenstockii	+	-
Achnanthidium sp.	+	-
Amphipleura pellucid	+	-
Amphora veneta	+	-
Actinella sp.	+	+
Amphora ovalis	+	+
Amphora sp.	*A*	+
Asterionella sp.	+	-
Audoeinella sp.	+	-
Aulocosiera sp.	+	+
Brachysira serians	+	-
Brachysira sp.	+	+
Caloneis sp.	+	+
Cavinula sp.	+	-
Cocconeis placentula	+	-
Cocconeis sp.	+	+
Coconeis pediculus	+	+
Craticula sp.	+	-
Cyclotella sp.	+	+
Cymbella affinis	+	-
Cymbella aspera	+	-
Cymbella cistula	+	-

Taxa	Undisturbed Sites	Deweeding Sites
Cymbella cymbiformis	+	-
Cymbella microcephala	+	-
Cymbella sp.	*A*	*A*
Cymbella tumida	+	-
Diatoma heimale	+	+
Diploneis sp.	+	-
Encyonema prostratum	+	-
Encyonema sp.	+	+
Entomoneis alata	+	+
Entomoneis sp.	+	-
Epithemia argus	+	-
Epithemia sorex	+	-
Epithemia sp.	+	+
Epithemia turgid	+	+
Eunotia sp.	+	-
Fragillaria capucina	+	-
Fragillaria sp.	*A*	*A*
Fragillariforma virescens	+	-
Fragillariforma sp.	+	-
Frustulia rhomboids	+	-
Frustulia sp.	*R*	+
Gomphoneis sp.	+	*R*
Gomphonema accuminatum	+	-
Gomphonema auger	+	-
Gomphonema olivaceum	+	+
Gomphonema purvulum	+	-
Gomphonema sp.	+	+
Gomphonema truncatum	+	-
Luticola heufleriana	+	-
Melosira sp.	+	-
Mastogloia sp.	+	-
Meriodon circulare	+	-
Meriodon sp.	+	-
Navicula cryptocephala	+	+
Navicula digitoradiata	+	+
Navicula lanceolatum	+	-
Navicula menisculus	+	+
Navicula cincta	+	+
Navicula phyllepta	+	-
Navicula sp.	*D*	*D*
Neidium binodis	+	-

Taxa	Undisturbed Sites	Deweeding Sites
Nitzschia palea	+	+
Nitzschia paleacea	+	-
Nitzschia pusilla	+	-
Nitzschia hantzschiana	+	-
Nitzschia sp.	+	+
Pinnularia sp.	+	+
Pinnularis sp	+	-
Punctastrata sp.	+	-
Stauroneis anceps	+	+
Stauroneis phoenicenteron	+	-
Stauroneis sp.	*A*	-
Synedra acus	+	-
Synedra sp.	+	*A*
Synedra ulna	+	+
Synedra vaucheriae	+	-
Tabellaria sp.	+	*R*
Tryblionella debilis	+	-
Chlorophyceae		
Ankistrodesmus sp.	+	-
Ankistrodesmus falcatus	+	+
Ankistrodesmus spiralis	+	-
Pediastrum boryanum	+	-
Pediastrum biradium	+	-
Chlorella vulgaris	+	*A*
Cladophora sp.	+	-
Closterium acutum	+	-
Closterium sp.	+	-
Closteridium sp.	+	+
Cosmarium granatum	+	-
Cosmarium impressulum	+	-
Cosmarium renifome	+	-
Cosmarium sp.	+	+
Coelestrum sp.	+	+
Crucigenia sp.	+	+
Desmidium sp.	+	-
Kirchneriella sp.	+	+
Microspora sp.	+	+
Mougetia sp.	+	+
Oedogonium sp.	+	-
Pediastrum biradiatum	+	-
Rhizoclonium sp	+	-

Taxa	Undisturbed Sites	Deweeding Sites
Rhopalodia gibba	+	-
Scenedesmus communis	+	-
Scenedesmus armatus	+	+
Scenedesmus dimorphus	+	-
Scenedesmus obliquus	+	+
Scenedesmus quadricauda	+	+
Scenedesmus sp	+	-
Selenastrum westii	+	-
Selenastrum sp.	+	+
Spirogyra sp.	+	-
Stigeoclonium sp.	+	-
Straustrum sp.	+	-
Tetraedron hemisphaericum	+	-
Tetraedon sp.	+	-
Tetrastrum heterocanthum	+	-
Ulothrix sp.	+	-
Volvox sp.	+	-
Cyanophyceae		
Anabaena sp.	+	+
Calothrix braunii	+	-
Gleocapsa sp.	+	-
Lyngbya sp.	+	-
Merismopedia sp.	*R*	+
Microcystis aeroginosa	+	-
Oscillatoria sp.	+	+
Phormidium sp.	+	-
Xanthophyceae		
Vaucheria sp.	+	-

D = Dominant; A=Abundant; + present; R=Rare; - Absent

Sorenson's similarity coefficient

Based on the similarity between Undisturbed and Dredging sites, the value of Sorrensons similarity coefficient came 0.39 (39%), which means these sites differed by 61%.

Student's *t* test was applied between undisturbed and deweeding site. The results show significant difference (t value=17.35, $p<0.05$) between undisturbed and deweeding sites in terms of Periphyton abundance. There was considerably higher population abundance in undisturbed sites. ANNOVA was also applied on the abundance of Periphytic algae of undisturbed sites. There was insignificant ($F_{67,3}$ =0.495, P=0.68) variation among undisturbed sites. ANNOVA ($F_{7, 138}$ = 39.9, P=0.001) was also applied on other sites of the Lake ecosystem.

The Periphyton community recorded from undisturbed sites of the Lake did not show any significant variation; even the sequence of dominant taxa was almost identical. The reason for this structural similarity may be attributed to almost identical physico-chemical environment of these sites. As these sites remained untouched by dredging and deweeding operation, highest density was recorded.

When different natural substrates (macrophytes) of the lake were taken into consideration, significant variation was observed in the population density and dominance pattern of different taxa. This would suggest that the nature of the substrate has an important role to play on the size and composition of the Periphyton population. It has been observed that some of the dominant taxa occurred regularly on various substrates and none was strictly limited to any biotype. This might be due to their better adaptability (McIntire, 1971).

Maximum colonization on macrophytes was observed when the plant species entered the phase of senescence and started decaying towards the end of the growing season (Late Autumn). This may be because of the fact that the old parts of a macrophyte become soft and spongy due to decay and thus make a suitable substratum for the attachment of the Periphytic flora. It may also be due to rapid metabolic leakages from the decaying macrophytes. This is supported by Brix (1994), Wetzel and Allen (1972); Fontaine and Nigh (1983). The decreased populations during summer revealed the less abundance of most of the dominant Periphytic species and increased grazing pressure by zooplankton (Higgins *et al*., 2014).

M. Spicatum followed by *C. demersum* supported rich and varied Periphytic flora both in terms of number of taxa and the population density. This could be because of its greater surface area with highly dissected nature of its leaves. Similar findings were also observed by Harrod and Hall (1962) and Cyr and Downing (1988). According to Cattaneo and Kalff (1979), finely dissected plants like *Myriophyllum* have much greater biomass than coarsely leaved ones as the former have much larger surface area per unit weight than the latter. Some specific macrophytes like *T. natans and N. Peltata* harboured less number of Periphyton species. It may be due to increased release of allelopathically active compounds by this macrophyte that might have hampered the growth of Periphyton (Wium-Andersen *et al*., 1982).

The influence of deweeding on Periphyton was mainly due to habitat disturbance through removal of vegetation. Deweeding on one hand decreased the abundance of Periphytic algae (attached) and on the other increased the numerical abundance of phytoplankton (freely moving). This might be due to vigorous shaking of macrophytes by harvesters, the Periphytic forms got dislodged in the process, remained suspended and added to the overall numerical abundance of plankton group. Periphyton attached to surface of macrophytes competes with these plants for different nutrients (Underwood and Thomas, 1990) thus, harvesting of Periphyton infested macrophytes paves the way for growth of uninfested macrophytes which utilize the resources fully in absence of the Periphyton.

Decline in the number of Periphytic species during deweeding process can be attributed to the fact that the manipulated environment formed due to the removal of weeds does not provide the necessary requirements for the concerned Periphytic algae in appropriate capacity to flourish. This is supported by the fact that the reference site which doesn't undergo any deweeding does not exhibit any significant changes in the Periphytic algae during the same period.

The harvesting operation at some sites was found to favour the growth of invasive macrophyte species (Engel, 1990). The re-growth of macrophytes is possible in two ways. Firstly the underground parts that were left behind during harvesting grew new shoots quickly. Secondly, the viable fragmented portions of stem, stolon or rhizome, cut from the parent plant during deweeding form the nucleus of new population. Besides, the harvester may also spread the propagules (fragment, buds and turion) throughout the lake (Habib and Yousuf, 2014).

Due to harvesting, apical dominance of macrophytes was hampered. This probably promoted the release of cytokinin plant hormone that led to denser and accelerated growth of lateral branches (Cline, 1994), thereby hampering apical dominance. Besides, rapid re-colonization of macrophytes occurred perhaps through vegetative fragments (Vári, 2013) that were dispersed during partial deweeding early in the season (Engel, 1990; Nichols and Lathrop, 1994).

Prior to deweeding, the Periphytic community was observed to be composed of a few dominant and several less common and some rare taxa. But upon initiation of deweeding process, maximum number of dominant taxa got

removed along with the harvested weeds, which as a result gave an opportunity for some of the rare taxa to grow. Thus, as a consequence of which species composition and distributional pattern of this very important autotrophic community got altered. The present study therefore concludes that there was a negative impact on periphyton community by deweeding processes as evident by 67.15% loss in the species composition immediately after the initiation of harvesting operation. The recovery rate of studied Periphytic community was also registered to be slow. In order to ascertain the full recovery of Periphytic community, continuous long term assessment (>10 years) is mandatory.

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Effect of Arsenic on Certain Biochemical Parameters in Liver Tissue of an Air Breathing Fish *Channa gachua*

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Abstract

The environment is currently polluted by thousands of chemicals or xenobiotic introduced into the environment by man to meet the demands of the modern era. The pollution is continuous and alarming in flux to aquatic environment worldwide from both naturally occurring and anthropogenic sources. The polluted water may lead to the destruction of the beneficial species either directly effecting aquatic forms of life in directly through breaking the biological food chain such as fish and their habitat and behavioral pattern. The fish as a bio indicator of aquatic medium plays an important role in the monitoring of water pollution because of the sudden death of fish indicates heavy pollution and the effects of exposure to sub lethal levels can be measured in terms of biochemical, physiological and histological responses of the fishes. In the present study, the sub lethal effects of arsenic on various biochemical parameters of *Channa gachua* were studied. The fish was exposed to sub lethal concentration of arsenic for 20 days for chronic toxicity studies. Total protein, amino acid and acetyl cholinesterase, glycogen and lactic acid were observed. The present study showed the protein content was decreased and amino acid content was increased significantly and also Acetyl cholinesterase was increased in the liver tissue of arsenic treated fish, *Channa gachua*. The present study shows the level of glycogen decreased and lactic acid increased in the liver tissue of fish exposed to arsenic. These changes were concentration dependent.

Keywords: Biochemical parameters, arsenic, liver, *Channa gachua.*

Introduction

Arsenic is a trace element and it under goes multiple electron transfer reactions. Arsenic exists in soluble and insoluble forms, organic and inorganic trivalent and pentavalent forms among which the trivalent arsenite is highly toxic. It occurs in the earth's crust along with sulphides and iron pyrites. The sources of arsenic found in environment includes natural and manmade. It is released into the human environment including drinking water through the mining and burning coal, smelting of copper and through industrial effluents. Chemicals containing arsenic are also used in the manufacturing of herbicides and pesticides, leads shots and phosphate detergents and in preservation of wood and hide. Arsenical herbicides and pesticides applied to agricultural soils and vegetation also may be important sources of arsenic contamination of food stuffs respectively. Arsenic is a toxic element for humans and it is commonly associated with serious health disruptions (Brookes, 1998). Total diet as studies carried out in various countries have shown that fish and shell fish are the most significant dietary source, accounting for nearly three quarters of total intake (Dokkun *et al.*, 1989

and Tao *et al.,* 1999). The concentration of arsenic was found in environmental samples, mainly in waters where inorganic form is predominant (Smith *et al.,* 2000, Elci *et al.,* 2008). Arsenic exposure has been related to the appearance of some types of cancer (Tchounwou *et al.,* 2003). A report on an assessment of the cancer risk associated with consumption of oysters caused a panic among consumers in Taiwan (Guo, 2002). Some of these human health effects currently observed in population of South and South eastern Asia, particularly in countries such as Bangladesh and India (Al Ramali *et al.,* 2005). Besides the direct exposure of humans as through drinking contaminated water, this might also be biologically available to aquatic organisms, such as fish which are used as human food there by providing an additional source of nutrition. Arsenic has a considerable tendency to accumulate in bottom sediments (Svobodovo *et al*., 2002). For this reason, issues related to as content in aquatic organisms and sea fish in particular, have attracted considerable attentions. The relevance of this arsenic intake will depend on the concentration of accumulated by the fish (Lai *et al.,* 2001). During recent years, serious concern has been voiced about the rapidly deteriorating state of fresh water bodies with respect to toxic metals pollution. Fishes are often at the top of the aquatic food chain and accumulate large amounts of some metals from the water (Tuzen, 2003).Water pollution leads to fish contamination with toxic metals from many sources such as industrial and domestic wastewater, natural runoff and contributory rivers (Rashed, 2001 and Tariq *et al.,* 1991). Fishes, living in polluted water may accumulate toxic trace metals via their food chains; they assimilate metals by ingestion of particulate material suspended in water, ion exchange of dissolved metals across lipophilic membranes, e.g., the gills, adsorption on tissue and membranes surfaces. The bioaccumulation of metals is therefore, an index of the pollution status of the relevant water body (Alam *et al.,* 2002).

Protein is the most important and abundant biochemical constituent present in the animal body. Hence, Proteins are important in all biological systems. Protein and amino acids are very important nutrients. Protein plays a major role in the synthesis of microsomal detoxifying enzymes and helps to detoxify the toxicants which enter into the animal body (Ramasamy, 1987). Amino acids are the building blocks of protein which are organic compounds, meaning that they contain carbon and hydrogen bonded to each other. In addition to those two elements, they include nitrogen, oxygen and in few cases sulfur. The basic structure of an amino acid molecule consists of a carbon atom bonded to an amino group which is the ($-NH_2$), a carboxyl group (-COOH) a hydrogen atom, and a fourth group that differs from one amino acid to another and often is referred to as the -R group or the side chain. The R-group, which can vary widely, is responsible for the differences in chemical properties of fetus (Sankar and Jagadeesan, 2006). Acetyl cholinesterase is an enzyme present in various tissues, including muscle and red cells, that breaks down acetylcholine a chemical released by nerves that activates muscle contractions and helps to maintain proper transmission of impulses between nerve cells and between nerve cells and muscles; also called true cholinesterase. Measuring acetyl cholinesterase in amniotic fluid may help confirm a suspected neural tube defect in the foetus Sankar and Jagadeesan (2006). Carbohydrate is an essential energy source for all vital activities of an organism. It is stored in the form of glycogen in animals. Glycogen is broken down into glucose for energy requirements. The stressful condition disturbs the metabolic rate of carbohydrate and thus the level of glycogen; glucose and lactic acid are altered (Srivastava and Singh, 1980; Metelev *et al.,* 1983; Rahman and Shamim 2014). The present study was carried with an aim to investigate effect of the sub lethal dose of arsenic in biochemical parameters in liver tissue of an air breathing fish *Channa gachua.*

Materials and Methods

Live specimens of *Channa gachua* were procured from local fish dealers at Hazaribag (Latitude 25º 59'N and Longitude 85º 22'E) and maintained in large glass aquaria size (90 x 60 x 60 cm) with continuous flow of water. The specimens were fed on chopped goat liver daily during a minimum acclimation period of 20 days in the laboratory. Routine oxygen consumption from air and still water was measured in a closed glass respirometer containing 3 litres of water (initial O_2 content = 6.5 mg O_2/ litre; pH = 7.2) and 0.51 mL of air. The fish were acclimated to the respirometers for at least 12 hours before the readings were taken. The experiments were conducted at 29.0 ± 1.5°C. The pH of the ambient water was measured by an electronic pH meter systronics. The respiratory chambers were thermostated by immersion in a temperature controlled water bath. Fishes from 70-100 grams were used during the experiment. They were checked thoroughly for injury and disease conditions and only healthy fishes were used for this study. After washing with 0.01% $KMnO_4$ solution for 15 min, they were placed in nine plastic pools (300 L) containing non chlorinated water. Prior to the start of the experiment the fishes were acclimatized to the food and laboratory conditions with 12 hours dark and 12 hours light cycles. Fishes were divided into five equal groups each comprising of 30 fishes. Each group was kept in separate aquarium tanks. The first group was kept as negative control; the fishes were maintained in water containing normal water without any treatment. The fishes of two groups were exposed to a sub lethal concentration of 1 ppm concentration of Arsenic added in the water for 30 days respectively. Solutions were renewed once daily after exposure period, animals were sacrificed and the liver tissues were removed, homogenized and stored at −80 °C for further biochemical analysis. Protein content in the tissue was estimated by the method of (Lowry *et al.,* 1951). Total free amino acids and content of the tissue were estimated by the method of (Moore and Stein, 1954). The glycogen content was estimated by Kemp and Kits (1954) and Lactic acid was done by the method of Barker and Summerson (1941). The data were subjected to student "t" test to find out the significance of difference between control and treated values.

Results and Discussion

In *Channa gachua* the gills are pinkish red in colour. Each gill arch is made up of two primary gill filaments. The filaments are beset with the secondary gill lamellae. The gills receive blood from the afferent branchial artery near the origin of gill lamellae. The opening of efferent branchial artery is situated just below the afferent branchial artery. The transverse ligament present between the both arteries. In the present study, attempts have been made to investigate the effects of sub lethal concentration of arsenic on various biochemical parameters of *Channa gachua* in acute and toxicity studies. In the liver tissue of control groups, the protein content was 86.92±1.98 mg/g wt. wt. of tissue. After the mercury exposure the level of protein content was significantly decreased in liver tissue of arsenic exposed fish, as compared to respective control levels (Table 1). The level of protein content was increased in arsenic exposed fish. In the liver tissue of control fish, the acetyl cholinesterase activity was 45.72±0.95 moles of acetylcholine hydrolysed per mg of protein per hr. During the arsenic exposure the activity of acetyl cholinesterase was decreased in the liver tissue of fish. The level of glycogen content in the liver tissue of control fish was 11.99±1.96 mg/g wet wt. of tissue. During the arsenic exposure the level of glycogen decreased in the liver tissue (8.42±0.97mg/g wet wt. of tissue) in the liver tissue of control groups respectively. The lactic acid content was 2.84±1.08 mg/g wt. wt. of tissue. After the arsenic exposure the level of lactic acid content was significantly decreased in liver tissue of arsenic exposed fish, as compared to respective control levels.

Table 1: Showing biochemical parameters in liver tissue of *Channa gachua* treated with arsenic.

Parameters	Control	20 days treated
Protein (mg/g)	86.92±1.98	73.85±1.68*
Amino acid (mg/g)	2.85±1.54	3.66±1.87*
Acetyl cholinesterase (AChE)	45.72±0.95	36.14±1.92*
Glycogen	11.99±1.96	8.42±0.97*
Lactic acid	2.84±1.08	4.22±1.87

Mean ±S.D of six individual observations; *significance at 5% level

In the present study a reduction in the protein content observed in *Channa gachua* exposed with arsenic. These results suggest that the tissue protein undergoes proteolysis results in an increase in the production of free amino acids. These amino acids are utilized for energy production during stressful situation in the intoxicated fishes. Neff (1985) has reported that decline in protein content may also be related to increased energy cost of homeostasis, tissue repair and detoxification during stress. In the present investigation sub lethal concentrations of arsenic exposed fish *Channa gachua* exposed with arsenic show a decrease in protein content and an increase in amino acid content of liver for 20 days exposure of arsenic. Many investigations have also reported such a change in total protein content of various tissues in different fishes exposed to different heavy metals (Rajamanikam,1992 and Pazhanisamy, 2002). Jana and Bandyopadhyay (1981) have reported such a reduction in protein content when the fish *Channa punctatus* has been exposed to heavy metals such as mercury, arsenic and lead. Protein depletion has been reported in the liver of *Anabas testudineus* exposed to nickel chloride (Jha and Jha, 1995). Decrease in the liver protein level is reported in the fish *Labeo rohita* exposed to arsenic Pazhanisamy (2002) *Channa punctatus* exposed to zinc and phenyl mercuric acetate (Sen *et al.,* 1992 and Karuppasamy, 2000) *Channa punctatus* exposed to arsenic (Jatyajit, 1996) *Channa striatus* exposed to mercury cadmium and lead (Palanichamy and Baskaran, 1995) and *Cirrhina mrigala* exposed to lead acetate (Ramalingam *et al.* 2000). Baskaran *et al.* (1989) have reported the impact of commercial detergent Nirma on feeding energetics and protein metabolism in the fresh water teleost fish *Oreochromis mossambicus*. The decrease in liver and muscle protein has been reported in the sugar mill effluent treated *Channa punctatus* after 96 hours exposure (Avasan and Ramakrishna, 2000). In the present investigation, the decreased level of protein in brain tissue shows that fish exposed to arsenic are subject to stress. Similar results have also been recorded in the protein content of different tissues when the animals are exposed to various pollutants (Palanichamy *et al.,* 1989; Malla and Basha, 1988; Manoharan and Subbiah 1982. Meenakshi and Indra (1998) have noticed depletion in the level of total protein in liver and muscle and an increase in the total free amino acids in blood, liver and muscle of distillery effluent treated *Mystus vittatus*. Anuradha and Raju (1996) have observed the increased level of amino acid content in liver, muscle, kidney and gill tissues of *Anabas scandens* exposed to selenium toxicity. The FAA serves as metabolites for a TCA cycle which have a key role in stepping up the energy requirement respectively.

Acetyl chollinesterase (AchE) activity measurement in fish has been used for monitoring the neurotoxicity of pesticide (Bretaud *et al.,* 2000). AchE, a serine hydrolase catalyzes the breakdown of the neurotransmitter acetyl choline into acetate and choline. This process involves the formation of a substrate enzyme complex, followed by acetylation of the hydroxyl group, the amino acid serine, present within the eastertic side and finally deacetylation. The inhibitory effect on AchE activity indicates that pollutants like insecticide might

interfere in the vital processes like energy metabolism of nerve cells (Nath and Kumar, 1999). AchE inhibition and an accumulation of ACh in the tissues of sumithion treated fish *Channa gachua* have been observed by Kaundinya and Ramamurthy(1978). Basha and Sailbala (1989) have observed a steep decline in AchE activity with a concomitant elevation in AchE content in different tissues like gill, kidney, brain, liver and different types of muscles in *Cyprinus carpio* following 10 days exposure to malathion. The decrease in brain AchE is found to be inversely proportional to the increase in Ach content in methyl parathion treated tadpoles of frog, *Rana cyanophiclits*. Ravi and Selvarajan (1990) have reported an increase in the levels of amine in the brain region of *Labeo rohita* and *Cyprinus carpio* exposed to phosalone. Sevgiler *et al.* (2004) have reported a significant correlation between increase in lipid peroxidation and inhibition of AchE activity in liver. They have further stated that etoxazole mediated lipid peroxidation may be related to its anticholine esterase action. Increased lipid peroxidation caused by etoxazole indicates that this compound induces the generation of reactive oxygen species, creating oxidative damage in the cell membrane. Yang and Dettbarn (1996) in their study with disisopropyl fluorophosphates have suggested that AchE inhibitor induced cholinergic hyperactivity has initiated the accumulation of free radicals leading to lipid peroxidation, which may be the initiator of AchE inhibitor induced cell injury. Nachmanson and Feld (1947) have reported that the animal dies when AchE activity of the brain is inhibited by 95 percent. Coppage (1972) have observed 79 percent reduction in AchE activity in the esturine fish *Lagodon rhomboids* exposed to 48 hours median lethal concentrations (92±g/L) of malathion. Carbohydrate is an essential energy source for all vital activities of an organism. It is stored in the form of glycogen in animals. Glycogen is broken down into glucose for energy requirements. The stressful condition disturbs the metabolic rate of carbohydrate and thus the level of glycogen; glucose and lactic acid are altered (Srivastava and Singh, 1980; Metelev *et al.*, (1983). The toxic substances are absorbed into the body and transported to various organs through blood. The blood glucose is a sensitive biochemical indicator of stress. Exposure of fishes to different types of toxic substance is known to elicit changes in the biochemical constituents and thereby altering the metabolic pathways. In the present study the level of glycogen content and lactic acid was increased in the liver tissue of fish exposed to arsenic. Changes in the glycogen level of liver have been noticed by many investigators. Mcleay and Brown (1974) have recorded a considerable decrease in glycogen content of bleached kraft pulp mill effluent. Baskaran *et al.*, (1989) have noticed the depletion on the hepatic glycogen content in *Oreochromis mossambicus* when exposed to textile dye effluent. Depletion in the glycogen content of liver and muscle has been observed in *Rasbora daniconius* exposed to pulp and paper mill effluent (Vijayaram and Vasugi; 1989). *Channa gachua* exposed to sub lethal concentration of arsenic shows an overall increase in the blood glucose at all periods of exposure thereby indicating that the glycol genolysis takes place in the liver, where by the reserved glycogen is being slowly converted into glucose. The hyper glycemic condition in the present study correlated with the observations of some researcher's *viz.*, the juvenile Coho salmon on *Oncorhynchus kisutch* treated with sub lethal concentration of neutralized unbleached kraft mill effluent Mcleay (1973). Similar results were made by Vijayram and Vasugi (1989) in paper and pulp mill effluents. Similar elevated blood glucose levels have been noticed in *Heteropneustes fossilis* exposed to textile mill effluent Nisha and Shukla (1986). Lactic acid is formed through glycolysis under anaerobic condition of glucose catabolism. In the present study it showed an increase in the lactic acid content of liver and blood at all the hours of effluent treatment. Accumulation of lactic acid is more in liver and blood of fishes exposed to raw effluent. It is likely that the lactic acid formed in the muscle and other tissue during glycolysis, might have been transported to liver *via* blood accounting for the hyper lactamia in blood and liver. Because of the absence of the enzyme glucose 6-phosphatase in the

muscle which is necessary for the conversion of lactic acid into glucose, the lactic acid produced in the tissue is transported to the liver through blood (Shanmugam, 1980). Since liver is the metabolic site the lactic acid transported from the tissue to liver is utilized for the resynthesize the of glucose and glycogen through Cori cycle contributing to the increase in the level of lactic acid in liver and blood at all periods of study. Burton *et al.* (1972) have observed the heavy accumulation of lactic acid in liver of rain brown trout *Salmo gairdneri* exposed to zinc. Rahman and Shamim (2014) reported that impact of zinc sulphate on bio chemical parameter in *Channa gachua* reported that the decreased glycogen concentration in the liver could be due to its enhanced utilization as an immediate source to meet the energy demand under metallic stress through glycolysis or hexose monophosphate pathway. It is assumed that decrease in glycogen content may be due to the inhibition of hormones which contribute to glycogen synthesis. Depleted glycogen level under other heavy metals stress is also supports our findings with other workers. The increased in glucose level of tissue while decrement in tissue glycogen in exposed fish *Channa gachua* makes it clear that glycogen reserves are being used to meet the stress. To summarize these results indicate that the heavy metal at sub lethal and lethal concentrations altered the bio chemical composition of the test fish due to utilization of biochemical energy to counter act the toxic stress due to heavy metals present in effluents.

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Micronucleus Test in Erythrocytes of *Cyprinus carpio*: A Sensitive Monitor for Aquatic Pollution

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Abstract
Micronuclei test is a system of mutagencity testing used for determining the pollution and chemicals causing changes in DNA fragments such as micronuclei in the cytoplasm of interphase cells. Damage caused on the DNA by genotoxic pollutants is the first consequence occurring in the aquatic organisms. The aquatic environment makes up the major part of our environment and resources, therefore its safety is directly related to the safety our health, thus, it was attempted to determine whether pollution affected the erythrocytes of fish *Cyprinus carpio* at the level of DNA by the means of micronuclei (MN) test. The test has been used successfully as a mutagenic assay. It is simple, reliable, sensitive, and it does not depend on any karyotypic characteristics. Fish were collected from locations that display differential environmental stresses. Organisms used in the MN test were collected from Dal Lake and Mansbal Lake. According to the results of the present study, frequency of MN was found higher in fishes from Dal water compared to Mansbal Lake. In conclusion, this study indicates that the micronuclei test gives sensitive results in monitoring the pollution, and thus it might be used as standard method in regular monitoring of pollution of water bodies.
Keywords: Fish, mutagenicity, micronucleus assay, erythrocyte

Introduction

Ecosystems are undergoing unprecedented alterations in their indices due to climate changes, biogeochemical cycles, and changes in land use and hydrology (Bogoni *et al.*, 2014). Metabolic processes in most aquatic environments are supported by debris and land materials contributing to the energetic stability and production of organisms. Thus, understanding changes in the dynamics of the matter transported to the riverbeds is particularly important to understand several processes (Kominoski and Rosemond, 2012). The contamination of surface waters, containing known and unknown compounds, could pose a serious public health and aquatic ecosystem threat (Claxton *et al.*, 1998). This increased the interest in studies for the evaluation of polluted water genotoxicity. In recent years several studies have evaluated the impact of agricultural and industrial effluents on river waters using different assays (Lemos and Erdtmann, 2000; Vargas *et al.*, 2001; Vigano *et al.*, 2002; Tagliari *et al.*, 2004; Ohe *et al.*, 2004; Ergene *et al.*, 2007 and Lemos *et al.*, 2007). Anthropogenic activities as sources of increased toxic substance content in aquatic systems are now common in Kashmir.

Aquatic biota is constantly exposed to great number of toxic substances during their lifespan both from the water and through aquatic food chain (Dar *et al.*, 2015). Studies reveal the fact that a number of chemicals contaminating the environment have carcinogenic or mutagenic effects. The major sources for the mutagenic and carcinogenic substances are industrial and agricultural activities (Bogoni *et al.*, 2014). Xenobiotics from these sources ultimately contacts the aquatic ecosystems. Although many hazardous substances exist in the water and sediment and they are accumulated by aquatic organisms and triggers DNA or cellular damage and even affects the ecosystem by passing through the tropic chain (Izquierdo *et al.*, 2003).

In recent years increasing concern about genotoxic pollution in water bodies has led to the development of many different mutagenesis test systems. Water and sediment samples can be tested for mutagencity under laboratory conditions using biological systems such as bacteria, yeast and plants (Minissi *et al.*, 1996 and Ergene *et al.*, 2007). The use of fish as bio-indicators of pollutant effects is being more and more used since fishes are very

sensitive to changes in their environment and play significant roles in assessing potential risk associated with contamination in aquatic environment of new chemicals (Dar *et al.*, 2015 and Nwani *et al.*, 2010); they are frequently used test organisms for studying cytotoxicity, water toxicity and genotoxicity. For example, at cellular level the micronucleus test on various fish tissues is among the most widespread assessments of genotoxicity in water (Ansari *et al.*, 2011 and Arkhipcuk and Garanko, 2005). Micronuclei (MN) test is one of the most reliable techniques used to determine genetic changes in the organisms. MN experiments is a fast method in detecting the chromosomal damage because it makes possible to determine the remaining chromosomes and broken chromosomes due to its several advantages such as (a) giving more objective results than other tests in detecting chromosomal impairments, (b) being easy to learn, (c) it does not require to count the chromosomes to investigate the chromatids and chromosomal damage hard to detect and see in the metaphase stage, (d) its preparation stage is fast and (e) it makes it possible to count thousands of cells, not hundreds of cells in each experiment (OECD, 2014). For all these reasons, the micronucleus test in fish erythrocytes seems to be promising test in environmental mutagenesis investigations (Al-sabti and Metcalfe, 1995).

In the present paper, micronucleus frequency in fish erythrocytes has been evaluated in *Cyprinus carpio* from fresh water environment characterized by different pollution levels, and compared with the values observed in erythrocytes of fish collected from less polluted water body. The aim of the present study was to validate the sensitivity of this test system and the suitability of bioindicators in environmental monitoring. As indicator species, *Cyprinus carpio* was chosen because of its ecology, wide distribution in fresh water environment of Kashmir, availability throughout the seasons, easy acclimatization in the laboratory conditions and commercial importance make this species as an excellent test specimen for geno-toxicological studies by comparing among different lakes. These considerations have prompted interest in the development of such techniques and its use as bioindicators for monitoring the genomic damage from environmentally hazardous contaminants in the aquatic environment.

Materials and Methods

Collection sites and sampling

The valley of Kashmir is situated in the middle of the Himalayas between the northwest and southeast (33°01′–35°00′N latitude and 73°48′–75°30′ E longitude) at an altitude ≥1500 m above sea level. The study was carried out in Dal Lake (34°07′ N, 74°52′ E), and Mansbal Lake (34°15′ N, 74°40′E). The Dal Lake is an urban lake that lies to the east of Srinagar city, at the foot of Zabarwan Hills, and is situated at an average elevation of 1,583 m (5,194 ft) above sea level with a maximum depth of 6 m (20 ft). Mansbal Lake is a rural lake situated at a distance of 32 km from Srinagar city. Its length and breadth are approximately 3.2 and 1 km, respectively. The lake is situated at the altitudinal zone of 1,585-1,600 m (5,200-5,200 ft) with a maximum depth of 13 m (43 ft). Recent studies have attested that the Dal lake has reached to the level of eutrophic condition, but the level of trophic state varies, with Dal lake being the most eutrophic and Manasbal Lake being the least nutrient enriched (Zargar *et al.*, 2012). *Cyprinus carpio* L. (Family: Cyprinidae and Order: Cypriniformes), was selected as the test organism. Live juvenile specimen, procured with the help of cast net from both the sites, were transported to the laboratory and subjected to a prophylactic treatment by bathing in 0.05% potassium permanganate ($KMnO_4$) for 2 min to avoid any dermal infection. Their average length and wet weight (±SD) were recorded as 18.12±0.62 cm and 77±6.782 g, respectively for Dal lake and 17.62±1.70 cm and 66.25±17.97 g, for Mansbal Lake.

Experimental procedures

Two main experiments were carried out. In the first one, blood samples were directly collected from caught fish. In the second experiment, fish were acclimated for a week in 60 L glass aquaria with well aerated water at 20°C. The specimen maintained in dechlorinated tap water and then exposed to Ethyl methanesulfonate (5 mg/L, concentration selection based on previous investigation; Cavas and Konen, 2008) were considered as the positive controls.

Micronucleus test

Peripheral blood samples were obtained by caudal vein puncture using heparinised syringe. Blood was immediately smeared on clean, grease free frosted glass slides. Slides were fixed in methanol for 10 min and left to air dry at room temperature and finally stained with 6% geimsa in Sorenson buffer(pH 6.9) for 20 min. MN were

identified and scored microscopically under 100 X in an Olympus microscope. Two thousand and five hundred erythrocytes were scored for each specimen to determine the frequency of micro nucleated erythrocytes. Slides were scored by a single observer using blind review. For MN scoring purpose, only non refractive small nuclei (>1/3 of the main nucleus) located close to the main oval nucleus of round erythrocytes with intact cytoplasm were considered (Schmid, 1975; Das and Nanda, 1986 and Ali *et al.*, 2009).To avoid intra specific differences related to fish size for each species, only adult specimens with similar sizes were sampled.

Results and Discussion

The obtained results are summarized in Tables 1 & 2 and Figure 1. Results reveal that the fish represent various degrees of sensitivity in monitoring genetic damage (especially clastogenic effect). The present results indicate that micronuclei frequencies were low in fish from Mansbal compared to that of Dal Lake. In the positive control experiments, the frequency of micro nucleated erythrocytes from fish treated with EMS was significantly higher in *C.carpio*.

This is indicated by variations in averages of the micronucleated cells among species at different locations. As previously mentioned by different limnologists these locations display differential environmental stress. These lakes were categorized according to approved international standards (OECD, 1982). As the valley is devoid of chemical factories nutrient input from domestic sewage define the main source of pollution. The marked difference in the water quality of the two lakes clearly depicts the influence of anthropogenic stresses on the lakes. These results are also in confirmation with the loading concept and support the findings of Pandit and Yousuf (2002).

Table 1: Length and weight of fishes collected at monitoring sites and scored for micronuclei

Sites	Length(cm) (mean±S.D)	Weight(g) (mean±S.D)
Dal	18.12±0.62	77±6.782
Mansbal	17.62±1.70	66.25±17.97

Table 2: Mean micronuclei counts for *C. carpio* from the two monitoring sites.

Sampling locations	No. of fishes observed	No. of cells observed (*NEA*)	MN frequencies (%)± SE	PC
Dal Lake	5	12500	0.15±0.007	0.14±0.08
Mansbal Lake	5	12500	0.08±0.06	0.21±0.08

NEA No. of erythrocytes analyzed; PC positive control

The contamination of aquatic environments poses serious consequences for the welfare of the organisms exposed because pollutants may induce mutations and cancer (Beyersmann and Hartwig, 2008). Chromosomal mutation is an important occurrence in carcinogenesis (Fenech, 2000). The presence of micronuclei represents a parameter for determining the extent of damage caused by an environmental agent to the process of cell division of the affected tissue (Vine, 1990) and reveals threats that cannot be detected through chemical or physical analyses. Cytogenetic methods are probably the most sensitive and effective means by which to detect the effects of genotoxins (Bogoni *et al.*, 2014).

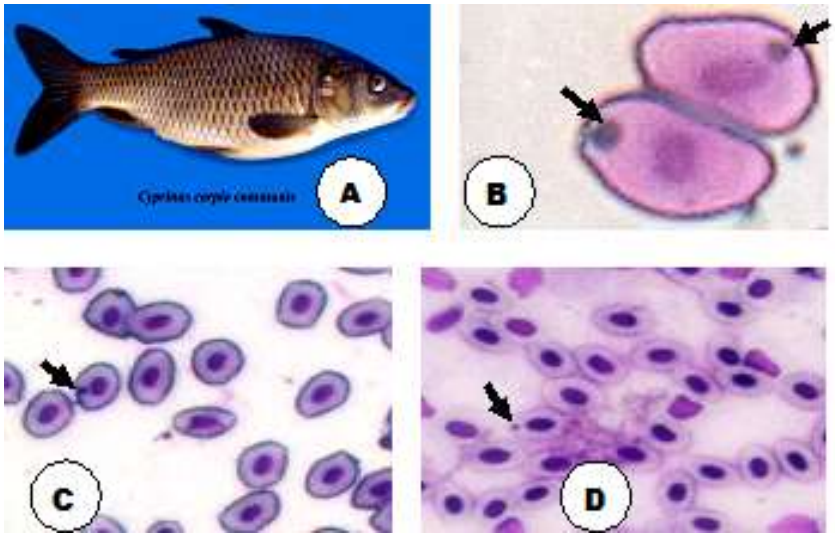

Figure 1: Photomicrograph showing *C.carpio* **(A)** and micronucleated erythrocyte **(B, C & D).**

Fish serve as sentinel organism for ecotoxicological studies because they play a number of roles in tropic web, accumulate toxic substances and respond to low concentration of mutagens. Therefore, the use of fish biomarkers as indices of the effects of pollution, are of increasing importance and can permit early detection of aquatic environmental problems (Nwani *et al.*,2010). Villela *et al.* (2006) and Al-Sabti and Metcalfe (1995) showed that fish are excellent subjects for the study of the mutagenic and/or carcinogenic potential of contaminants present in water samples since they can metabolize, concentrate and store waterborne pollutants. The sampling of peripheral blood is appropriate and sufficient for biomonitoring projects. Lyne *et al.* (1992) and Cesar and Célia (2000) demonstrate that *Tilapia rendalli* and *Oreochromis niloticus*, give different responses to genotoxic agents. According to clastogen and the species studied, the frequency of micronuclei may suffer important variations. Time-dependent responses have also been observed in amphibians exposed to radiation. Siboulet *et al.* (1984), Fernandez *et al.* (1993) and Ali *et al.* (2009) confirmed that the micronuclei frequencies may vary according to the season, the kind of pollution involved and the species of fish. It was concluded from these studies that fish can be used for estimating the genotoxic effects of water-borne pollutants.

Therefore, based on the results obtained, the main finding is that under the conditions and period of this study, the waters of the Dal lake induced genetic damage in erythrocytes of *C.carpio* because the frequency of MN was greater compared with the that of the Mansbal. Different references showed that MN can be affected by many factors such as age, sickness, species, feeding, chemical and physical agents and environmental conditions (Al-Sabti and Metcalfe, 1995; Saleh and Zeytinoglu, 2001). So to eliminate these factors, healthy, young and Active individual had been chosen from the same species, *C. carpio*.

The obtained results support the fact demonstrated by Kligerman (1982) that fish inhabi ting polluted waters have greater frequencies of micronuclei. The most remarkable result is that MN frequency appears to be strongly related to water quality of the different environments examined. The relationship between MN frequency and pollution levels observed in fresh water fish reflects what already observed by different authors in marine fish from coastal areas(Hose 1994), beside being in accordance with that observed by means of in situ exposure of rainbow trout to polluted riverine waters (Deflora *et al.*,1993). The presence of different pollutant in the waters of Dal Lake was evidenced by Zargar *et al.* (2012). On the contrary, the low MN frequencies observed in the Mansbal Lake lead to the conclusion that in this lake genotoxic agents are not detectable.

Discharging very low concentrations of chemicals, such as PAHs and trace metals (e.g., Cu, Zn, Pb, Mn, and Fe), into the environment may affect all levels of biological organization, from the molecule to the ecosystem. The duration of time from the moment of introduction of a contaminant into the environment until the very first

(harmful) physiological effects on the biota may vary between hours to decades (Everaarts *et al.*, 1998). Genotoxic chemicals may cause somatic and heritable mutations. Somatic mutations cause cellular damage that can ultimately result in disease. Such stress can reduce viability, survivability, and reproductive success. Heritable effects, such as deleterious germ-line mutations, also produce these effects. Subsequently, the genetic makeup of populations might be altered by the reduction of genetic variability, the increase of deleterious alleles, or the fixation of low-frequency alleles as the population becomes adapted to new environmental conditions (Bickham, 2000).

The results of the present investigation on the genotoxic potential of the polluted water of Dal lake suggested a serious concern about its potential danger to aquatic organisms, especially to fish, and indirectly to human beings. However, further studies are needed to explore the biological consequences of DNA damage in aquatic organisms due to deleterious effects of polluted water of Dal lake and to formulate future strategies for safeguarding aquatic organisms and environment.

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Contribution of Anganwadi Centers of Ganderbal District (J & K), in Promoting Health Awareness and Preparedness for Primary Schooling: An Evaluative Study

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Abstract

India is the home of the largest child population in the world. The development of children is the first priority on the country's development agenda, not because they are the most vulnerable, but because they are our supreme assets and also the future human resources of the country. Tenth Five Year Plan (2002-07) underlines the fact that the future of India lies in the future of Indian children- across income groups, geographical locations, gender and communities. Researches on women and children reveal that there are several areas which require the attention of planners and programme implementers. Policy decisions based on research findings are rooted in ground reality and therefore have the capacity to bring about tangible improvement in the situation whether it is with regard to nutritional status, health practices or rights of women and children. The present study was undertaken to study the contribution of anganwadi centers in promoting health awareness and to study their contribution in preparing of children for primary schooling. In the present research, descriptive survey method and purposive sampling technique was used by the investigator to collect data from 40 Anganwadi workers (AWW), 40 beneficiaries (mothers) from Ganderbal district of Jammu and Kashmir. Self developed interview schedule for beneficiaries and a questionnaire for AWW was used to study the contribution of anganwadi centers. Descriptive statistics was used to describe the main features of collected data in quantitative terms .The results of the study of Ganderbal district revealed that food is provided to each category of beneficiaries and is provided regularly however the quality of food served is not satisfactory. Weight of children is also measured regularly and growth cards are also maintained. Immunization is provided to beneficiaries and immunization cards are also maintained. Malnourished and disabled children are also detected through Anganwadi centres (AWCs). Learning activities are conducted, teaching aids are also used by AWW during the conduction of learning activities. Information is also provided to children by AWW regarding the benefits of education. Mothers are aware about the enrollment of their children in AWC however children are not attending the AWC regularly. The quality of food served in AWC is not satisfactory and child weight is not also being measured regularly. No home visits are done by health staff during pregnanancy and mothers were not very satisfied with the services provided by anganwadi centers in preparing the children for primary schooling.

Keywords: Anganwadi workers, Anganwadi Centres, Integrated Child Development Services

Introduction

Children are the most valuable section of our society. No nation on this globe can ignore the responsibility to ensure their proper growth and development as the future of the country lies with them. All the children neither have equal opportunities and facilities for living and learning nor have the same level of social acceptability. Developmental programmers aimed at reducing poverty do not necessarily reach children or improve the environment in which they live and grow. As per 2001 census the country has around 17% of children who are below the age of 6 years and majority of them live in economic and social environments which could impede the child's physical and mental development. These conditions include poverty, poor environmental sanitation, disease

infection, inadequate access to primarily health care, inappropriate child caring and feeding practices. The National Policy for Children was adopted in 1974 and the Integrated Child Development Services (ICDS) scheme was launched as a sequel to it in 1975. The National Policy for Children, 1974, has been adopted on the conviction that child development programmes are necessary to ensure equality of opportunity to all children. It provides the framework for assigning priorities to different needs of children (both before and after birth) and for responding to them in an integrated manner. ICDS is India's response to the challenge of meeting the holistic needs of the child (Gupta *et al.,* 2005).

The ICDS scheme was sponsored by the Government of India in 1975 with the major objective of providing opportunities of physical and psycho-social development to children in the age group of 0-6 years through an integrated package of early childhood services. The ICDS Scheme was launched with the objectives:

1. To improve the nutritional and health status of children in the age-group 0-6 years
2. To lay the foundation for proper psychological, physical and social development of the child
3. To reduce the incidence of mortality, morbidity, malnutrition and school dropout
4. To achieve effective co-ordination of policy and implementation amongst the various departments to promote child development and
5. To enhance the capability of the mother to look after the normal health and nutritional needs of the child through proper nutrition and health education.

Supplementary nutrition is the primary aspect of integrated child development services which includes supplementary feeding and growth monitoring and prophylaxis against vitamin A deficiency and control of nutritional anemia (George *et al*., 1993 and George *et al.*, 2000). Growth monitoring and nutrition surveillance are two important activities that are undertaken. Referral services include health check-ups and growth monitoring, sick or malnourished children, in need of prompt medical attention, are referred to the primary health centre or its sub-centre. The anganwadi worker has also been oriented to detect disabilities in young children. She enlists all such cases in a special register and refers them to the medical officer of the primary health centre/ subcentre.

Pre-school education is the sixth fundamental component of the ICDS programme, in which all its services essentially converge at the anganwadi a village courtyard (Saini and Sharma, 2002). Anganwadi Centre (AWC) a village courtyard is the main platform for delivering of these services. These AWCs have been set up in every village in the country. As per Census of India 2001, there are 157.86 million children below six years of age, and many of them have inadequate access to health care, nutrition sanitation, child care early stimulation, etc. To ensure that all young children, even those from vulnerable section of society have access to their basic right, ICDS provide a package of service to ensure their holistic development. ICDS provides health, nutrition, immunization, preschool education health and nutrition education, and referral services to young children and their mothers (Thimmayamma, 1987; Jindal, 1999; Manhas and Qadri, 2010). ICDS also empowers mothers of take better care of their children).

However, in spite of the expansion of ICDS, evaluation studies done by Forum for Creche and Child Care Services (FORCE) indicate that ICDS reaches out to 30% of the children. Children from remote scattered hamlets and children living in new slum clusters are often of ambit of ICDS services. Malnutrition has decreased only marginally from 47% in 1998-99 to 46% in 2005-06, as was revealed in the National Family Health Survey III (2006). The Supreme Court, in its order dated 29.04.2004 directed the Government of India to increase the number of AWC to cover 14 lakhs habitations. Efforts are being made to universalize ICDS so that a functional AWC exists in every settlement and full coverage of children is ensured.

During the Eleventh Five Years Plan (2007-2012), nutritionally backward would be the focus of special attention, and micronutrient supplementation /fortification would be used as a strategy to combat specific micronutrient deficiencies. In spite of many lacunas in the functioning of the scheme, the achievement under ICDS are many. Notable among them is the progressive decline in infant and child mortality, and the spread of awareness about

immunization and health and nutrition education. Research reveals the true ground realties and field situation, and as a pointer to the path which needs to taken to achieve desired results (Tyagi and Pradhan, 2015).

Singhi *et al*. (1996) evaluated the strengthening quality and access to services in ICDS programme in Rajasthan. The findings of the study were reflected that there was need to strengthen the integration of ICDS with other departments like health and education. community should be involved to decentralize services like supply of supplementary food and other resources (Gopalan, 1992; Gopalan and Ramasastri, 1993). The AWW should have decision making power, receive adequate teaching material and equipment, and undergo reorientation training at regular intervals. Close and supportive relationship between gram panchayat and AWCs should be established. There is need to have variation in the food served to match the taste of children. Vacant posts should be filled. Awareness building campaigns through local communication mechanisms should be initiated. Gender compatibility should be established through the involvement of both, men and women. Serious thought should be given to raise the salary of AW helper.

Sobha (2003) Conducted study on Welfare services for women and children in Tirupati. The findings of the study reflected that it was suggested that improvement in service conditions' AWWs, frequent in-service training, incentives for better work in achieving better results, supply of essential medicines, strengthening of health and nutrition education to AWWs, supply of teaching aid and toys in AWCs, and supply of good quality weaning food should be ensured. Efforts should be made to spread awareness about ICDS scheme through mass media and personal contacts.

Kapil (2001) conducted study in Rajasthan to assess the weight gain pattern and nutrient intake among 61 severely malnourished children during different seasons. The study recommended that extra nutritional care should be provided to young children during summer and rainy seasons, as their growth and nutrient intake is comparatively lower in these seasons than during the winter season (Gopal, 2008)

The Centre for North East Studies and Policy Research (CNESPR) conducted a study to assess the functioning of ICDS in the AWs of Assam and Meghalaya. There were many complaints against the ready to eat (RTE) packets as these were half opened and damaged. In several villages, pregnant women also refused immunization. Many parents did not allow their children to be weighed because of superstitio. Community must be made aware of the benefits provided by AWCs. Services of ICDS should be available for every child under 6 years, not only for those from BPL families. Take-home rations (THR) for children should be provided on a regular basis.

Vinnarasan (2007), conducted a study on the factors influencing non- enrollment of children in ICDS AWC run by Chennai Corporation. 88 AWC situated in Adyar, Besant Nagar, Mandaiveli, Santhome, Kotturpuramand Pattinapakkam were covered. It was found that 47.3% respondents believed that the purpose of existence of the AWC was to look after young children. It was suggested that adequate funds should be allotted to improve the physical infrastructure of AWC and provide them with basic facilities. Training for the staff should emphasize the value of their work, impart skills to mobilize community support, and also sensitize them about the Right to Participation of children in AWC. Government should emphasize and strongly enforce the convergence of services to children through different departments. The focus of ICDS should shift to providing quality Preschool education as the main task, with nutrition and health services playing roles similar to the Mid Day Meals Scheme in schools (Jindal and Shipra. 1999; Saiyed and Seshadri, 2000; Manhas and Qadri, 2010). Nazam (2013) conducted a study on India's response to challenge of meeting the holistic needs of child. Today the Integrated Child Development Services is one of the world's largest and most unique outreach programs for early childhood care and development having completed three decades. It is one of the program in the world which not only addresses health, nutrition and development needs of young children, adolescent girls and pregnant and nursing mothers across the life cycle (Nair and Radhakrishnan,2004; Nair and Mehta, 2009).

Materials and Methods

Since the nature of the problem involved exploring and trying to understand the totality of a phenomenon in context- specific settings, a descriptive survey method was employed with purposive sampling technique. Two

blocks were selected from district Ganderbal. From each block twenty AWC were selected to make a total sample of forty AWW for the study. One mother was also selected from each center to make a total sample of forty mothers. Self-constructed questionnaires were developed for AWW, self-constructed Interview schedule was developed for mothers to get information regarding the contribution of Anganwadi centers for promoting health awareness and preparedness for primary schooling. The questionnaire and the interview schedule was divided into two parts (1) Promotion of health awareness (2) Preparedness for Primary schooling. In health awareness part the questions were framed on supplementary nutrition, growth monitoring, immunization, health check-ups and referral services while as in preparedness for primary schooling part questions were framed on pre school education. The validity of the questionnaire and interview schedule was established through face validity and content validity methods. The investigator visited CDPOs and sought their approval for collection of data. Descriptive statistics was used to describe the main features of collected data in quantitative terms. Children below the age of three years of age are weighed once a month and children 3-6 years of age are weighed quarterly. Weight-for-age growth cards are maintained for all children below six years. This helps to detect growth faltering and helps in assessing nutritional status. Besides, severely malnourished children are given special supplementary feeding and referred to medical service.

Result and Discussion

Results pertaining to the contribution of anganwadi centers in promoting health awareness. Response of AWW regarding the kind of food provided to each category in district Ganderbal.

Out of 40 respondents (AWW) of district Ganderbal 70% responded that they provide biscuits to infants and 30% responded that they provide khichdi to infants. 60% responded that they provide chanapulow and 40% responded that they provide Halwa to the toddlers. Pregnant women: 65% responded that they provide both khichdi and halwa and 35% responded that they provide channapulow.

Response of AWW regarding the adequacy of food served in Ganderbal District.

Out of 40 respondents 75% agreed that food is adequately served, where as 25% agreed that food is not adequately served.

Response of AWW regarding Quality of food served in AWC of District Ganderbal.

Out of 40 respondents 35% responded that the quality of food served is good, 15% responded that the quality of food served is poor, 40% responded that quality of food served is average and 10% responded that they cannot say about the quality of food served.

Response of AWW regarding the regularity of food provided to children in AWC of district Ganderbal.

Out of 40 respondents 70% responded that food served is very regularly , 30% responded that the food is served regularly, 10% responded that food served is somewhat regular and 0% responded that it is very irregularly. Hence it may be concluded that food in AWC of Ganderbal is served regularly.

Response of AWW regarding the measuring of Childs weight regularly in AWC of district Ganderbal.

Out of 40 respondents 80% responded that Childs weight is regularly measured at AWC of district Ganderbal and 20% responded that Childs weight is not regularly measured. Hence it can be concluded that the Childs weight is regularly measured in AWCs of district Ganderbal.

Response of AWW regarding the maintenance of Childs weight-for-age growth cards in district Ganderbal.

Out of 40 respondents 80% responded that they maintain Childs weight-for-age growth cards and 20% responded that they do not maintain Childs weight-for-age growth cards. Hence it may be concluded that the Childs weight-for-age growth cards are being maintained in district Ganderbal.

Response of AWW regarding to whom immunization is provided in AWCs of district Ganderbal.

Out of 40 respondents 20% responded that they provide immunization to pregnant women, 30% responded that

they provide immunization to infants, 50% responded that they provide immunization to both pregnant women and infants. Hence it may be concluded that immunization is being provided in district Ganderbal.

Response of AWW regarding the kind of immunization provided in AWC of istrict Ganderbal.

Out of 40 respondents 10% responded that they provide BCG, 15% responded that they provide DPT , 60% responded that they provide polio/measles and 15% responded that they provide immunization to all. Hence it may be concluded that polio/measles immunization is provided regularly in AWCs of district Ganderbal.

Response of AWW regarding the maintaining of immunization cards in district Ganderbal.

Out of 40 respondents 90% responded that they maintain immunization cards and 10% responded that they do not maintain immunization cards. Hence it may be concluded that immunization cards are being maintained by AWW in district Ganderbal.

Response of AWW regarding the health check- ups done in AWC OF District Ganderbal.

Out of 40 respondents 15% responded that the health check ups are given to children below 6 years, 20% responded that health check ups are given to expectant mothers, 10% responded that health check ups are given to nursing mothers and 55% responded that health check ups are given to all.

Response of AWW regarding the conduction of NHE sessions in AWC of district Ganderbal.

Out of 40 respondents 70% responded that they conduct NHE sessions with mothers and 30% responded that they do not conduct NHE sessions with mothers. Hence it may be concluded that NHE sessions are being conducted in AWC of district Ganderbal.

Response of AWW regarding the detection of malnourished and disabled children through AWC of district Ganderbal.

Out of 40 respondents 40% responded that they detect the malnourished and disabled children and 60% responded that they do not detect the malnourished and disabled children. Hence it may be concluded that the detection of malnourished and disabled children is not done to a great extent through anganwadi centers of district Ganderbal.

Results pertaining to the contribution of anganwadi centers in preparedness of children for primary schooling.

Response of AWW regarding the conduction of any learning activity in AWC in district Ganderbal

Out of 40 respondents 80% responded that they conduct learning activities and 20% responded that they do not conduct any learning activity. Hence it may be concluded that learning activities are being conducted in AWC of district Ganderbal.

Response of AWW regarding having of teaching learning equipments in AWC in district Ganderbal.

Out of 40 respondents 60% responded that they have teaching learning equipments and 40% responded that they do not have teaching learning equipments. Hence it may be concluded that teaching learning equipments are present in AWC of district Ganderbal.

Response of AWW regarding the using of teaching aids in AWC in district Ganderbal.

Out of 40 respondents 80% responded that they use teaching aids and 20% responded that they do not use teaching aids. Hence it may be concluded that teaching aids are used adequately in AWC of district Ganderbal.

Response of AWW regarding the providing of any information to children about the benefits of education in district Ganderbal.

Out of 40 respondents 95% responded that they provide information about the benefits of education and 5% responded that they do not provide. Hence it may be concluded that the information is being provided to children about the benefits of education.

Response of beneficiaries regarding their Child enrollment in district Ganderbal.

All the 40 respondents responded that they are aware about their Childs enrollment. Hence it may be concluded that children are enrolled in AWC of district Ganderbal.

Response of beneficiaries (mothers) regarding the children attending the AWC in district Ganderbal.

Out of 40 respondents 50% responded that their children attend the AWC regularly, 40% responded that their children attend the AWC occasionally and 10% responded that their children attend the AWC rarely. Hence it may be concluded that the children do not attend the AWC regularly in district Ganderbal.

Response of beneficiaries (mothers) regarding who motivated them to enroll their child in AWC in district Ganderbal.

Out of 40 respondents 50% responded that they have enrolled their child in AWC by their own motivation, 40% responded that AWW have motivated them and 10% responded on the motivation of health worker.

Response of beneficiaries (mothers) regarding the quality of food served in AWC in district Ganderbal.

Out of 40 respondents 10% responded that the quality of food provided is good, 70% responded that the quality of food provided is fairly good and 20% responded that they cannot say. Hence it may be concluded that food served in AWC of district Ganderbal is not good.

Response of beneficiaries (mothers) regarding the measuring of Childs weight in AWC in district Ganderbal.

Out of 40 respondents 20% responded that their Childs weight is regularly measured at anganwadi centers and 80% responded that their Childs weight measured occasionally at anganwadi centers. Hence it can be concluded that Childs weight is not regularly measured in AWC of district Ganderbal.

Response of beneficiaries (mothers) regarding having of immunization cards of their child in district Ganderbal.

Out of 40 respondents 95% responded that their children have immunization cards and 5% responded that they do not have.

Response of beneficiaries (mothers) regarding any home visits by health staff during pregnancy of district Ganderbal.

Out of 40 respondents 5% responded that health staff visited their home during their pregnancy and 95% responded that health staff did not visit their home during their pregnancy. Hence it can be concluded that the health staff does not visit the homes of beneficiaries in district Ganderbal.

Response of beneficiaries (mothers) regarding any type of counseling provided by AWW after visiting their homes in district Ganderbal.

Out of 40 respondents 15% responded that AWW provided them the counseling but 85% responded that they did not receive any type of counseling. Hence it may be concluded that AWWs in district Ganderbal do not provide counseling to beneficiaries.

Results pertaining to find out the views of beneficiaries (mothers) about the contribution of AWC in preparedness for primary schooling.

To achieve the above objective the research question, ‘‘ How beneficiaries view the contribution of AWC in preparedness for primary schooling has been framed? To respond the question, the data has been presented in subsequent paragraph.

Response of beneficiaries (mothers) regarding any learning activity organized for children at AWC in district Ganderbal.

Out of 40 respondents 40% responded that learning activities are organized by AWC, 20% responded that no learning activity is organized and 40% responded that they are not aware about this thing. Hence it can be concluded that to some extent learning activities are organized.

Response of beneficiaries (mothers) regarding learning activities are they beneficial for child in district Ganderbal.

All the 40 respondents responded that learning activities are beneficial for the child. Hence it can be concluded

that the beneficiaries are aware about the benefits of education.

Response of beneficiaries (mothers) regarding the attitude of AWW towards children in district Ganderbal.

Out of 40 respondents 85% responded that the attitude of AWW towards children is kind and 15% responded that they were unable to observe. Hence it can be concluded that attitude of AWW towards children is kind.

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Status of DNA Barcoding of Coccinellidae (lady bird beetles), Trichogrammatidae and Syrphidae (Hover Flies)

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Abstract

DNA barcode is a genetic signature that occurs naturally within the genome of every living species. One of the gene regions commonly used for all animal groups is a 648 base pair region in the mitochondrial cytochrome oxidase 1 gene (CO1), it has been effectively used in identifying birds, flies, butterflies, fishes and many other animal groups due to the high polymorphisms among species. Paul Herbert (2003) published a paper entitled "Biological Identification through DNA barcodes, which created awareness among scientists on the usefulness of DNA Barcode as an effective technique for identification of species. In the one decade of research after this publication, DNA barcode has evolved rapidly into a tool that can be employed for solving many environmental, agricultural, health and conservation problems around the globe. It also has applications in disease and pest control, market fraud detection and protection of endangered species. This paper reviews current status of DNA Barcoding of Coccinellidae, Trichogrammatidae and Syrphidae.

Keywords: Coccinellidae, trichogrammatidae, syrphidae, DNA barcode, mitochondrial cytochrome oxidase 1 gene (CO1)

Introduction

Coccinellids or ladybird beetles belonging to the family coccinellidae, order coleoptera are the most commonly known of all beneficial insects. The family name comes from its genus, *Coccinella*. Adult ladybird beetles are dome shaped, oval or convex, often shiny with short legs and antennae. Coccinellids are commonly yellow, orange or scarlet with small black spots on their wing covers. Such color patterns vary greatly; however, for example, a minority of species, such as *Vibidia duodecimguttata*, a twelve spotted species has whitish spots on a brown background.

Trichogrammtids belong to the family Trichogrammatidae under super family Chalcidoidea (Hymenoptera). These are minute chalcid wasps, endoparasitic and mostly egg parasitoids of more than 200 insect pests belonging to the order Lepidoptera, Coleoptera, Hemiptera, Neuroptera, Diptera, etc. Some also attack on the eggs of spiders and mites. So far more than 80 genera under the family Trichogrammatidae are reported from the world over.

The members of family Syrphidae are also called hover flies as the adults are seen hovering over the flowers for collection of nectar. Hoverflies provide crucial ecosystem services as pollinators, biological control agents and in environmental assessment (Mengual and Thompson 2011). The larvae have a wide spectrum of feeding habits, being Phytophagous, mycophagous, zoophagous and saprophagus. Some of the Syrphid maggots are insectivorous, eating aphids, thrips, and other plant sucking insects. This is beneficial to gardens, as aphids destroy crops, and hoverfly maggots are often used in biological control.

Taxonomic identification of above mentioned families is male oriented. However, this classification has given rise to many controversies. The morphological identification of these families is done by the use of male genitalia in combination with more traditional characters of adult. Earlier, some taxonomists used these characters to identify these families but majority of these names are not valid now. The exact identification up to species, sub species level can be made only with the help of DNA barcoding.

DNA barcoding is the taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species or strain. Although several loci have been suggested, the most common locus of the animals includes the mitochondrial COI gene (Hebert *et al.,* 2003). It is a technique of sequencing a short fragment of the mitochondrial cytochrome oxidase subunit 1 (COI) gene. The standard sequence used for this purpose is approximately 648 base pair stretch of mitochondrial CO1 gene fragment amplified by the universal primers.

The classical use of morphological trait for species identification has several limitations. They include, for example, the misidentification of a taxon due to the phenotypic plasticity of the trait studied or the existence of cryptic taxa. Moreover morphological keys are sometimes only effective for a particular life stage or gender. Thus a high level of expertise is often required to correctly identify species with the accuracy required in ecological studies. In addition to the large specialized workforce needed to perform species identifications, morphological taxonomy has other serious challenges. In many species, there are also significant differences in morphology between the genders and different life stages. Cryptic species, by definition, are often impossible to identify by morphological characteristics alone. All of these obstacles in species identification often lead to incorrect identification. Another reason for incorrect species identification is that some researchers attempt to use keys without the appropriate level of expertise. Furthermore, some of the taxonomic keys in use are flawed and such keys are rarely revised since to do so is a major undertaking.

The DNA barcoding approach might correctly present the best solution for identifying species when their morphology is of limited use (Hebert *et al.,* 2003). DNA barcoding has been found promising in the rapid description of biodiversity. Besides adult DNA barcoding also helps in identification of larvae and pupae. DNA barcoding solves the problem of identification of sibling species, cryptic species and sub species. DNA barcoding helps in phylogenetic analysis thus leading to conservation and management.

In DNA barcoding 650 bp stretch of cytochrome oxidase gene 1 from the mitochondrial genome is used. The 650 bp long stretch lies on the 5' end of cytochrome oxidase gene 1. This region acts as a reference to delimit one species from other related ones (Hajibabaei *et. al.,* 2007). The Barcoding Project (Hebert *et. al.,* 2003) has the potential to revolutionize the process of species identifications and lighten the workload for the diminishing population of Taxonomists.

DNA barcoding of three economically important insect families

1. Coccinellidae

There are about 6000 known spp. of Coccinellidae (Coleoptera: Insecta) which are worldwide in distribution. The majority of coccinellid species are beneficial because of their predaceous nature, but some are injurious, being Phytophagous on agricultural crops. Ladybird beetles (Coccinellidae: Coleoptera) are important predators in natural and agricultural habitats and prey upon many economically important pests, including aphids, mealy bugs, scale insects, thrips, leaf hoppers, mites, and other soft bodied insects (Khan *et.al.,* 2009). They are beneficial for controlling the populations of aphids, scale insects and mealy bugs. *Adalia tetraspilota* (Hope) and *Hippodamia* (Adonia) *variegata* (Goeze) are the predominant species of coccinellids in agro-ecosystems of Kashmir valley (Khan *et.al.,* 2007). So far only 192 spp. have been barcoded belonging to about 33 countries (Figure 1).

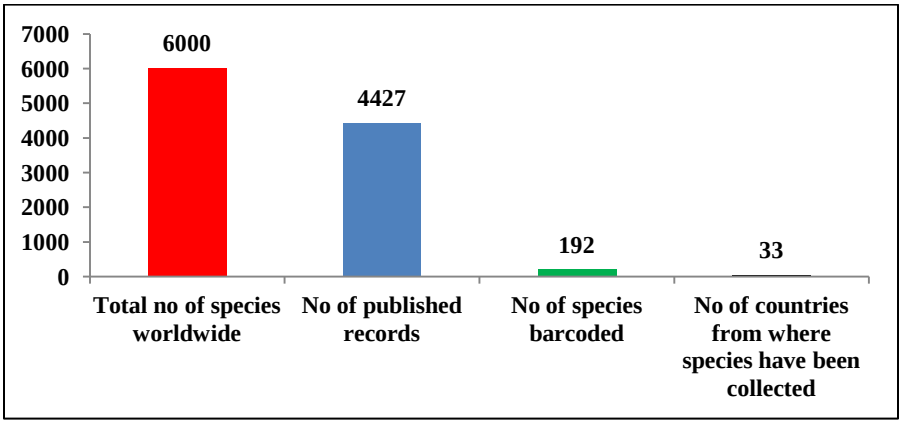

Figure 1: Showing details of DNA barcoding of Coccinellidae worldwide.

2. Trichogrammatidae:

Trichogramma is the most important genus under this family with over 200 species known from the world. Since all the species under this genus are exclusively egg parasitoids, hence constitute one of the widely used biological control agents against a number of serious insect pests throughout the world. The body length of *Trichogramma* ranges from 0.2 to 1.5 mm. They occur naturally all over the world, in almost every terrestrial habitat, and kill the pests in their egg stage by parasitizing them (Flanders and Quednau, 1960). Today, *Trichogramma* species are the most widely used insect natural enemy in the world, partly because they are easy to mass rear and they attack many important crop insect pests (Hassan, 1993). However, in most crop production system, the number of caterpillar eggs destroyed by native populations of *Trichogramma* is not sufficient to prevent the pest from reaching damage levels (Morrison *et.al.,* 1976). Apart from the potential use of different *Trichogramma* spp. against a number of insect pests attacking rice, maize, corn, cotton, apple, vegetables and pests of forest nurseries and plantations, throughout the world (Jalali and Singh., 1992; Hassan., 1989) their impact on the potentially insecticide resistant species, *Helicoverpa armigera* (Hubner). *Trichogramma* females oviposit inside the hosts eggs whose embryos are quickly killed and parasitized eggs turn black. This visible modification offers the opportunity to easily estimate female realized fecundity without waiting for offspring' emergence (Chiara *et.al*., 2012). The family Trichogrammatidae has nearly about 840 described spp. out of which only 25 spp. have been successfully barcoded belonging to 14 countries (Figure 2).

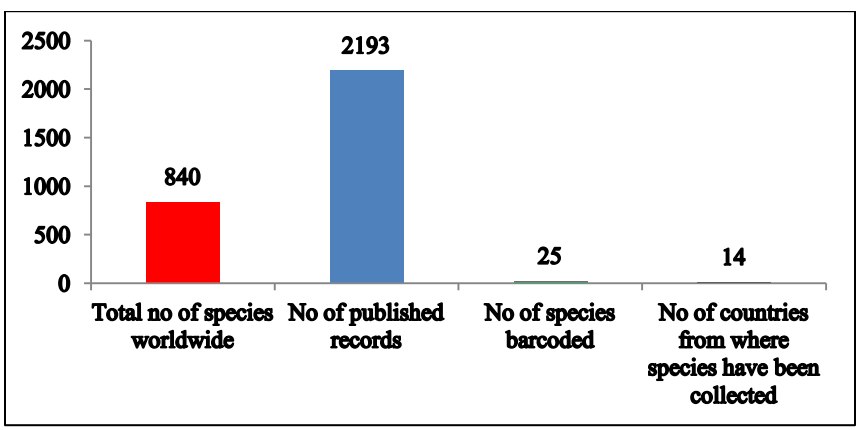

Figure 2: Showing details of DNA barcoding of Trichogrammatidae worldwide.

3. Syrphidae:

Syrphidae has a worldwide distribution, with almost 6,000 species described worldwide. Out of these 6000 spp. only 744 spp. have been barcoded so far which belong to 67 countries of the world (Figure 3). Hoverflies warrant attention not only because of their impressive diversity and economic importance, but also for their conspicuous habits and frequently eye-catching appearance (Miranda *et. al*., 2013).

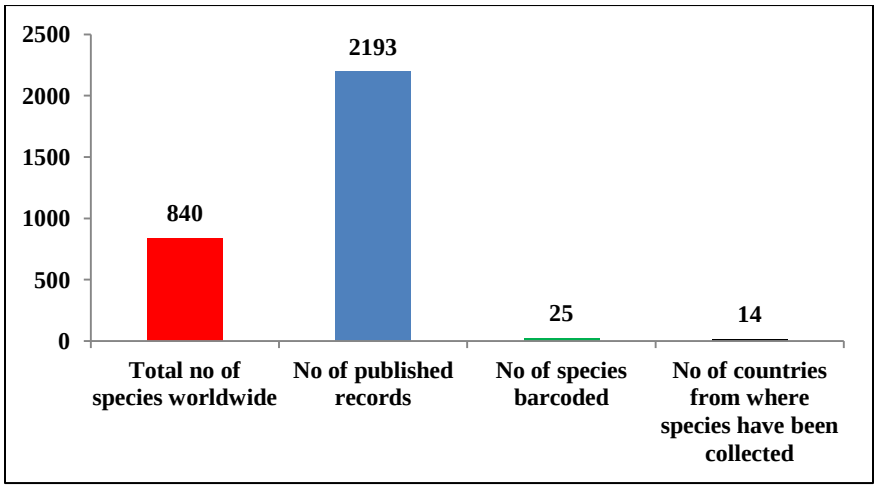

Figure 3: Showing details of DNA barcoding of Syrphidae worldwide

Various studies and analyses of those studies have been performed to determine the success of DNA barcoding for species identification. Meusnier *et al.* (2007) report barcoding Success levels over 97% in studies involving birds, mammals, fishes, and arthropods. Hebert *et al.* (2003) created a profile of one hundred species from seven diverse animal phyla and then attempted to identify newly analyzed taxa using this profile. This experiment resulted in a 96% success rate of correctly assigning the taxa to the appropriate phylum.

Conclusions

DNA barcoding has become a very important tool for species identification since its adoption in 2003. At present there are about one million species barcodes in BOLD system which is the official depository of DNA barcode data. iBOl, the major barcode project is likely to accelerate the creation of reference barcode libraries. If the DNA barcoding complements with the traditional taxonomy, it will be the major tool in species identification. The DNA barcoding should be supported because of its wide applicability in taxonomy and other branches of biology. DNA Barcoding is as important in developed countries as it is in developing nations for preventing the extinction of endangered species and poaching of our useful biodiversity such as medicinal plants and parts of wild mammals which are taken elsewhere, developed into useful products and exported back for trade at huge costs. With DNA barcoding, processed items can be identified and traced back to place of origin, thereby checking illegal trade and providing opportunity for benefit sharing among nations. Grants such as the Google Impact Award and awareness on the application of DNA barcoding would help developing countries with low technology in biodiversity conservation and management.

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Effects of Mutagenic Sodium Azide (NaN_3) on In-Vitro Development of *Nigella sativa*

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Abstract
The study examined mutagenic effects of Sodium azide (NaN_3) on micropropagation of *Nigella sativa* with or without growth hormones in MS medium. Seeds treated with NaN_3 (0.1%) for 6 hrs grown on MS medium containing various concentrations of Benzylaminopurine (BA) and Napthelene Acetic Acid (NAA) showed the best shoot number. It was observed that average number of shoots increased and the average shoot length decreased with increase in the BA concentration.
Keywords: Sodium azide, mutation, micropropagation

Introduction

Genetic variability is fundamental to successful breeding programs in vegetatively and sexually propagated plants. This variation can occur naturally or can be induced through mutations, using physical, biological or chemical mutagens and has attracted the interest of plant breeders for many decades. Mutations have been used to produce many cultivars with improved economic value (Broerties and Van Harten, 1988) and study of genetics and plant developmental phenomena (VanDenBulk *et al.,* 1992; Bertagne *et al.,* 1996). Mutations generally occur naturally (spontaneous mutation) but can also be induced by mutagens i.e. the physical or chemical biological agents that change the genetic makeup (Streisinger and Owen, 1985). Physical and chemical mutagens are more popular due to their cost effectiveness.

Seeds have high regenerative potential and are advantageous for use in mutagenesis. In vitro techniques can be used for both seed and vegetatively propagated species. Tissue culture techniques, combined with a mutagenesis treatment, speed up the breeding program. Chemically induced mutations generally lead to base pair substitutions especially GC→AT resulting in amino acid changes that change the function of proteins but do not abolish their functions as deletions or frame shift mutations mostly do (Veen, 1966). A common chemical used with seeds is the promutagen sodium azide, which must be metabolized by plant cells to the mutagenic agent presumably azidoalanine to be mutagenic (Owais *et al.,* 1983). The in vitro conditions help exposure of many varieties to mutagens easily as they can be exposed to mutagens in a relatively small space for reliable screening against mutations. Mutagens have been applied to suspension, callus and embryo cultures in many species including barley, soybean, carrot, maize, banana and morning glory (Blixt, 1965a; Blixt, 1965b; Blixt, 1967a; Blixt, 1967b; Broertjes and Lefferring, 1972; Kleinhofs *et al.,* 1978a; Kleinhofs *et al.,* 1978b; Bhagwat and Duncan, 1998; Bhate 2001). Successful use of mutagens requires optimum conditions to retain maximum germination capacity of seeds or adventitious shoot regeneration capacity of explants. Besides, the timing and dose of mutagen application are very critical and must be determined empirically. The aim of this study was to determine the optimum concentration and efficiency of in vitro Sodium azide (NaN_3) treated seeds of *Nigella sativa* and select mutated plants.

Materials and Methods

Filter sterilized solution of NaN_3 (1.5 M) was prepared in de-ionized water and diluted with sterile 0.1 M phosphate buffer (pH 3.6) to give 0.1 %, 0.2 %, 0.4% and 0.5 % working solution to treat the sterilized seeds. The seeds were given treatment for 6 and 24 hrs. The untreated seeds submerged in autoclaved distilled water for the same period of time

served as control. Prior to treatment with mutant all the seeds were sterilized by mercuric chloride (0.1%) for 10 minutes and then washed with autoclaved double distilled water. All the seeds were inoculated on MS basal medium (Murashige and Skoog, 1962) having 3% sucrose (carbon source) and 0.8% agar (solidifying agent) for germination. The pH of the medium was adjusted to 5.6-5.8 with 0.1 N NaOH or 1 N HCl before gelling with agar. The seeds were incubated in light intensity of 8/16 h (day/night) photoperiod.

The shoot tips of both treated and untreated seedlings were excised and inoculated on MS media supplemented with various concentrations of 6-benzyladenine (BA), kinetin (Kn) 2,4-Dichlorophenoxy acetic acid (2,4-D) alone or in combination with IBA or NAA. Each experiment was done three times in10–12 replicates. Data was recorded after 4 weeks of culture. The shoots obtained were inoculated on MS medium supplemented with different concentrations of IBA, IAA, NAA and 2,4-D to determine best rooting media. All the cultures were kept under cool-white fluorescent at 25±2°C and 60–70% relative humidity. The cultures were examined daily for contamination and morphogenetic responses and the data was recorded at the end of 4–12 weeks of culture period with respect to callus induction, its biomass, organogenesis (shoot and root induction) etc. The data collected on different parameters were subjected to statistical analysis to determine the degree of authenticity of results in terms of mean and standard error.

Results and Discussion

Seeds submerged in NaN_3 started germination after about 1 week of culture period. Seed germination rate decreased with increasing the mutagen concentration. The highest percentage of seed germination rate was observed at 0.1% NaN_3 solution for 6 hours. This response was almost equal to that of control used (Figure 1 & 2; Table 1). The complete germination of the seeds took eleven days with green leaves, epicotyl, hypocotyl, and root.

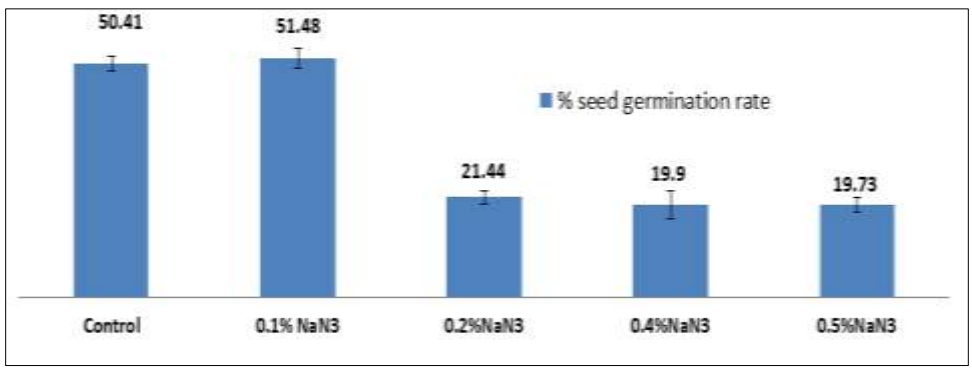

Figure 1: Percentage seed germination treated with sodium azide for a period of 6 hrs

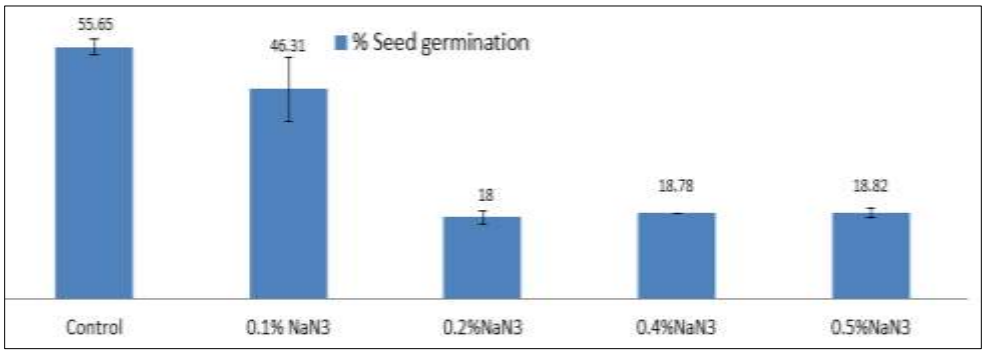

Figure 2: Percentage seed germination treated with sodium azide for a period of 24 hrs

Table 1: Effect of different concentrations of NaN_3 treatment for a period of 6 and 24 hours on seed germination of *Nigella sativa*

6 hours period		24 hours period	
Conc. of NaN_3	**Percent seed germination rate Mean ± S.E.**	**Conc. of NaN_3**	**Percent seed germination rate Mean ± S.E.**
Control	50.41±1.60	Control	55.65±1.67
0.1%	51.48±1.95	0.1%	46.31±7.03
0.2%	21.44±1.5	0.2%	18±1.41
0.4%	19.9±3.02	0.4%	18.78±0.02
0.5%	19.73±1.48	0.5%	18.82±0.83

Shoot tips obtained from both treated and untreated seeds were sub cultured in MS medium supplemented with a range of BA concentrations and the concentrations of 4.4µM, 5.55 µM and 8.8 µM resulted in shoot proliferation and callus growth (Figure 3). It was observed that average number of shoots increased and the average shoot length decreased with increase in the BA concentration (Table 2 and Figure 4).

Table 2: Shoot proliferation of Nigella sativa using MS medium supplemented with phytohormones

Phytohormone	**Callusing**	**No. of shoots Mean ± S.E***	**Length of shoots Mean ± S.E***
BAP(µM)			
4.9	+	1.8±0.4	3.62±0.57
5.5	++	2.0±0.5	2.25±0.41
8.8	+	2.67±1.0	1.67±0.27
Kn (µM)			
4.9	-	1.1±0.1	4.9±0.67
5.8	+	1.4±0.3	4.2±0.9
9.2	-	1.0±0.0	7.3±2.1
BA(6.66µM)+IAA(2µM)	++	2.4±0.6	2.17±0.28
BA(6.66µM)+NAA(2µM)	+	3.3±0.7	1.2±0.12
2,4-D(4 µM)+Kn(2µM)	+++	-	-
1/2MS+BA(4µM) + IBA(1µM)	++	1.67±0.19	1.6±0.1

The maximum average shoot number (2.67) was achieved on MS medium supplemented with BA 8.8 µM. A good callus and lateral growth was observed on MS +BA 5.55 µM while the shoot tips cultured in different concentrations of kinetin alone does not lead to any multiplication but lead to the elongation of shoot tips with a very scanty callus formation in some concentrations (Figure 3). The combined interaction of BA (6.66 µM) and IAA (2 µM) favored non regenerative callus formation with multiple shoot differentiation while BA (6.66 µM) and NAA (2µM) lead to the poor growth of callus (no regenerative) but a high multiple shoot differentiation. Highest average shoot number of 3.3 was achieved in this concentration. MS (half strength) augmented with BAP (4 µM) and IBA (1 µM) favored multiple shoot formation and good growth of callus. The shoot tips grew in size, shoot multiplication, callus growth was observed but there was no root induction.

Figure 3: Effect of different concentrations of cytokinins alone and in combination with auxins on multiplication of *Nigella sativa* [BA: a. 4.9 µM, b. 5.5µM, c. 8.8 µM; Kn: d. 4.9 µM, e. 5.8 µM, f. 9.2 µM, g. BA (6.6 µM) + IAA (2 µM), h. BA(6.6 µM) + NAA (2 µM), i. 2,4-D (4µM) + Kn (2 µM), j. ½ MS+BA(4 µM) + IBA(1 µM)

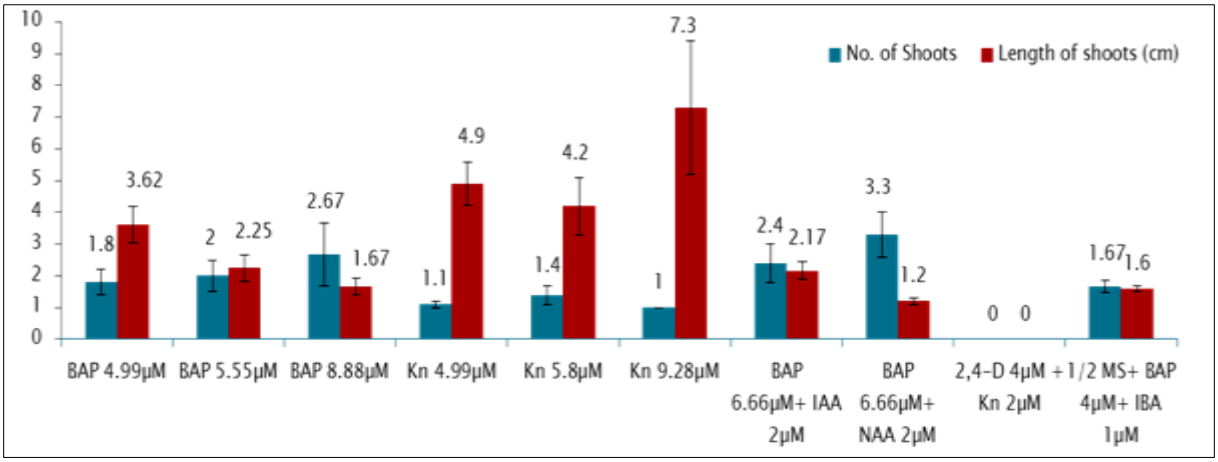

Figure 4: Effect of different concentrations of BA and Kinetin alone and in combination with IAA, IBA and NAA in shoot multiplication of *Nigella sativa.*

As far as effect of mutagenic treatment is concerned, in the present study it was observed that the shoot multiplication rate increased as the percent concentration of NaN_3 decreased and the maximum shoot multiplication rate was observed in the seeds treated with 0.1% NaN_3 for 6 hours. So our results revealed that we got a positive mutation which has improved shoot multiplication rate. Therefore using mutagenic NaN_3 (0.1% for 6 hrs.) produced mutants with possible improved characteristics.

The main purpose of in vitro propagation of plantlets is to develop complete plants from cells, tissues and organs or to develop new plants with new characteristics favorable to given environmental and climatic conditions. A lot of research is devoted in developing various mediums to suite the specific cultures. Numerous studies indicate that different cultures are successful in specific mediums i.e., the mediums vary in their composition from culture to culture. It is well known that organogenesis in vitro depends on a complex system of endogenous and exogenous interacting factors (Alicchio *et al.*, 1982). The regeneration ability may also vary from plant to plant depending upon the species and genotype, physiological conditions and type of explant.

The morphogenic response of the explant is mainly based on the type and concentration of hormone used. The combination effect of cytokinins and auxins to promote shoot induction and elongation has been reported from early studies. They are known to interact with different endogenous processes, including apical dominance, cell cycle, lateral root initiation, regulation of senescence, and vasculature development (Coenen and Lomax 1997; Swarup *et al.*, 2002).

In the present study the micropropagation of *Nigella sativa* was studied after treating the seeds with sodium azide. The study was conducted to observe the effect of mutagen on the micropropagtion rate as well as number of shoots. In vitro generated shoot tips were used as explants. Different phytohormones (auxins and cytokinins) were used individually and in combinations. In the present study, the combination effect of BAA and NAA was found most effective in inducing multiple shoot proliferation. Our results are in conformity of the results made by various researchers who had studied the combination effect of BAP and NAA (Khan *et al.* 1997; Hossain *et al.* 2003; Durkovic 2008; Frabetti *et al.* 2009; Girijashankar, 2011). Experimental observations revealed that combination of 2,4-D and Kn did not lead to the induction of shoots but a very good callus growth. Al-Ani (2008) also reported similar results. It was also observed that using BA alone also lead to the shoot proliferation and increasing the concentration of BAP in MS medium was found to increase the number of shoots while decreasing the average length of individual shoots. The maximum average shoot number of 2.67 was found in MS medium supplemented with 8.88 µM BA. In all the above cases, poor callus growth was observed. Using Kinetin (Kn) alone lead to the development of very low number of shoots but a very good shoot elongation and MS medium augmented with 9.28 µM kinetin showed maximum elongation of about 7.3 cm. A wider survey of existing literature reveals that BA is most reliable and useful cytokinin for multiplication of shoots (Barna and Wakhlu, 1994). BA is the most effective cytokinin for the shoot tip, meristem and bud culture. However Meyer and Staden (1991) and Natali *et al.* (1990) reported Kn better as compared to BA for shoot proliferation in Aloevera which is in contrast to our results.

The main objective of mutating plant species is to produce mutants with improved characteristics. Chemical mutagens especially sodium azide have been used extensively to produce mutants with improved characteristics (Kiruki *et al.*, 2006; Kumar, 1988; Ali *et al.*, 2007). In view of the above facts, seeds of *N. sativa* were given different mutagenic treatments of sodium azide and a dose dependent reduction in germination rate was observed i.e., increasing the NaN3 concentration (form 0.1-0.5%) decrease the % germination rate of seeds. Our results are in conformity with that of Bashir *et al.* (2013). Many workers have reported adverse effect of chemical mutagens on various plants (Koner *et al.*, 2007; Sangle *et al.*, 2011). The decrease in germination rate of seeds may be due to the damage of cell constituents at molecular level or altered enzyme activity (Khan and Goyal, 2009; Chowdhary and Tah, 2011). Therefore using mutagenic NaN_3 (0.1% for 6 hrs) produced mutant with possible improved

characteristics. Our results were supported by a number of studies which shows that NaN3 has been used in many plant species for improving their physiological characteristics (Suzuki *et al.*, 2008; Okubara *et al.*, 1993).

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Qualitative Phytochemical Analysis

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Abstract
Preliminary screening of phytochemicals is a valuable step in the detection of bioactive principles present in medicinal plants and may lead to novel environmentally friendly bioherbicides and drug. Plants are a source of large number of secondary metabolites comprising to different groups such as alkaloids, flavonoids, tannins, terpernoids, steroids etc. A large number of the plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another. Plants have been known to relieve various diseases in Ayurveda. Extraction of the bioactive plant constituents has always been a challenging task for the researchers. In this present review, an attempt has been made to give an overview of certain extractants and extraction processes.
Keywords: Secondary metabolites, alcoholic extraction, organic solvents, phytochemical screening

Introduction

There is ample literature on preliminary phytochemical surveys and the knowledge of the chemical constituents of plants is desirable to understand herbal drugs and their preparations. Most importantly, these studies will be helpful to isolate and characterize the chemical constituents present in those plant extracts. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Parray *et al.*, 2012).

Extraction is the separation of medicinally active portions of plant or animal tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form, and are intended for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts or powdered. extracts. Such preparations have been popularly called galenicals, named after Galen, the second century Greek physician (Harborne, 1984). Extraction methods involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity (Harbrone, 1984).

The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, countercurrent extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents). For aromatic plants, hydrodistillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed by distillation, expression and enfl eurage (cold fat extraction) may be employed. Some of the latest extraction methods for

aromatic plants include headspace trapping, solid phase microextraction, protoplast extraction, microdistillation, thermomicrodistillation and molecular distillation (Handa *et al.*, 2008).

The basic parameters influencing the quality of an extract are (Parray *et al.*, 2012):

1. Plant part used as starting material
2. Solvent used for extraction
3. Extraction procedure

Effect of extracted plant phytochemicals depends on (Parray *et al.*, 2012):

1. The nature of the plant material
2. Its origin
3. Degree of processing
4. Moisture content
5. Particle size

The variations in different extraction methods that will affect quantity and secondary metabolite composition of an extract depends upon (Harbrone, 1984):

1. Type of extraction
2. Time of extraction
3. Temperature
4. Nature of solvent
5. Solvent concentration
6. Polarity

Plant material

Plants are potent biochemists and have been components of phytomedicine since times of immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active components. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found (Parekh *et al.*, 2008). Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. Many authors have reported about plant extract preparation from the fresh plant tissues. The logic behind this came from the ethno medicinal use of fresh plant materials among the traditional and tribal people. But as many plants are used in the dry form (or as an aqueous extract) by traditional healers and due to differences in water content within different plant tissues, plants are usually air dried to a constant weight before extraction. Most researchers dry the plants in the oven at about 40°C for 72 h (Salie et al., 1996 and Gurjar et al., 20102). In most of the reported works, underground parts (roots, tuber, rhizome, bulb etc.) of a plant were used extensively compared with other above ground parts in search for bioactive compounds possessing antimicrobial properties.

Choice of solvents

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants (Eloff, 1998). The choice of solvent is influenced by what is

intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be nontoxic and should not interfere with the bioassay. The choice will also depend on the targeted compounds to be extracted (Parray *et al.*, 2012).

The various solvents that are used in the extraction procedures are:

Water: Water is universal solvent, used to extract plant products with antimicrobial activity. Though traditional healers use primarily water but plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extract. Also water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolics only important as antioxidant compound (Das *et al.*, 2010).

Acetone: Acetone dissolves many hydrophilic and lipophilic components from the two plants used, is miscible with water, is volatile and has a low toxicity to the bioassay used, it is a very useful extractant, especially for antimicrobial studies where more phenolic compounds are required to be extracted. A study reported that extraction of tannins and other phenolics was better in aqueous acetone than in aqueous methanol. Both acetone and methanol were found to extract saponins which have antimicrobial activity (Eloff, 1998).

Alcohol: The higher activity of the ethanolic extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts. It means that they are more efficient in cell walls and seeds degradation which have unpolar character and cause polyphenols to be released from cells. More useful explanation for the decrease in activity of aqueous extract can be ascribed to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts, whereas in methanol and ethanol they are inactive. Moreover, water is a better medium for the occurrence of the micro-organisms as compared to ethanol (Lapornik *et al.*, 2005). The higher concentrations of more bioactive flavonoid compounds were detected with ethanol 70% due to its higher polarity than pure ethanol. By adding water to the pure ethanol up to 30% for preparing ethanol 70% the polarity of solvent was increased (Bimakr, 2010). Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. Methanol is more polar than ethanol but due to its cytotoxic nature, it is unsuitable for extraction in certain kind of studies as it may lead to incorrect results (Wang, 2010).

Chloroform: Terpenoid lactones have been obtained by successive extractions of dried barks with hexane, chloroform and methanol with activity concentrating in chloroform fraction. Occasionally tannins and terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents (Cowan, 1999].

Ether: Ether is commonly used selectively for the extraction of coumarins and fatty acids [Cowan, 1999].

Methods of extraction:

Variation in extraction methods usually depends upon:

1. Length of the extraction period
2. Solvent used
3. pH of the solvent
4. Temperature
5. Particle size of the plant tissues
6. The solvent-to-sample ratio

The basic principle is to grind the plant material (dry or wet) finer, which increases the surface area for extraction thereby increasing the rate of extraction. Earlier studies reported that solvent to sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal.

Extraction procedures

Plant tissue homogenization: Plant tissue homogenization in solvent has been widely used by researchers. Dried or wet, fresh plant parts are grinded in a blender to fine particles, put in a certain quantity of solvent and shaken vigorously for 5 - 10 min or left for 24 h after which the extract is filtered. The filtrate then may be dried under reduced pressure and redissolved in the solvent to determine the concentration. Some researchers however centrifuged the filtrate for clarification of the extract (Parray *et al.*, 2012).

Serial exhaustive extraction: It is another common method of extraction which involves successive extraction with solvents of increasing polarity from a non polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted. Some researchers employ soxhlet extraction of dried plant material using organic solvent. This method cannot be used for thermolabile compounds as prolonged heating may lead to degradation of compounds (Nikhal *et al.*, 2010).

Soxhlet extraction: Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermolabile compounds as prolonged heating may lead to degradation of compounds (Nikhal *et al.*, 2010).

Maceration: In maceration (for fluid extract), whole or coarsely powdered plantdrug is kept in contact with the solvent in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermolabile drugs (Harbrone, 1984).

Decoction: This method is used for the extraction of the water soluble and heat stable constituents from crude drug by boiling it in water for 15 minutes, cooling, straining and passing sufficient cold water through the drug to produce the required volume (Mute, 2009).

Infusion: It is a dilute solution of the readily soluble components of the crude drugs. Fresh infusions are prepared by macerating the solids for a short period of time with either cold or boiling water (Roy, 2010).

Digestion: This is a kind of maceration in which gentle heat is applied during the maceration extraction process. It is used when moderately elevated temperature is not objectionable and the solvent efficiency of the menstrum is increased thereby (Audu *et al.*, 2007).

Sonication: The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like extraction of *Rauwolfia* root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules (Handa, 2008).

Percolation: This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an appropriate amount of the specified menstrum and allowed to stand for approximately 4 h in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstrum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h. The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional menstrum is added as required, until the percolate measures about three quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to the percolate. Sufficient menstrum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting (Handa, 2008).

Phytochemical screening:

Phytochemical examinations were carried out for all the extracts as per the standard methods.

Proteins (Xanthoproteic Test)

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins (Jigna and Sumitra, 2007).

Aminoacids

To the extract, 0.25% (w/v) ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid (Harborne, 1984).

Tannins

About 0.5 g of the dried powdered samples is boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride is added and observed for brownish green or a blue- black coloration (Jigna and Sumitra, 2007).

Saponins

About 2 g of the powdered sample is boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed constantly with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion (Harborne, 1984).

Flavonoids

5 ml of dilute ammonia solution is added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids (Harbrone, 1984).

Steroids

Two ml of acetic anhydride is added to 0.5 g methonlic extract of each sample with 2 ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids (Sofowara, 1993).

Cardiac glycosides (Keller-Killani test)

Five ml of extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Harborne, 1984).

Phenols (Ferric Chloride Test)

Extracts are treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols (Sofowara, 1993).

Carbohydrates (Molisch's Test)

Extracts are treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates (Harborne, 1984).

Alkaloids (Dragendroff's Test)

Plant extracts is treated with dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids (Harborne, 1984).

Conclusion

Non standardized procedures of extraction may lead to the degradation of the phytochemicals present in the plants and may lead to the variations thus leading to the lack of reproducibility. Efforts should be made to produce batches with quality as consistent as possible (within the narrowest possible range) and to develop and follow the best extraction processes.

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Phytochemical Screening of *Ajuga bracteosa* Wall ex. Benth: An Endemic Medicinal Plant of Kashmir Himalaya

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Abstract

Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. Many pharmaceutical agents have been discovered by screening natural products from plants. These natural products remain an important source of new drug leads and new chemical entities. Phytoconstituents obtained from plants have two categories i.e., primary and secondary. Primary constituents include chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids and alkaloids. Medicinal plants have antifungal, antibacterial, antioxidant, antimutagenic and anti-inflammatory activities because of the presence of these phytoconstituents. Therefore, the present study was taken to have a preliminary investigation of the various phytoconstituents from the crude extracts of *Ajuga bracteosa* known as Jani- Adam. The crude extract showed the presence of various phytoconstituents like alkaloids, phenolics, tannin, cardiac glycosides, terpenes, flavonoids, saponin, steroids, carbohydrates and proteins. It is expected that the important phytochemical constituent recognized in *Ajuga bracteosa* found in Kashmir Himalaya will be very useful in the curing of various diseases of this region.

Keywords: Phytoconstituents, *Ajuga bracteosa*, antimutagenicity, EMS, micronucleus

Introduction

Traditional herbal medicine practitioners have described the therapeutic efficacy of many indigenous plants (Bharat and Parabia 2010). The plants are the source of synthetic and traditional herbal medicine and hence are useful for healing and curing of human diseases because of the presence of phytochemical constituents (Nostro *et al.* 2000; Rao and Savithramma 2012; Choudhary *et al.* 2013). These phytochemicals are naturally present in all parts of medicinal plants viz. leaves, vegetables and roots. Phytochemicals are primary and secondary metabolites which are synthesised by the plants itself. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds include terpenoid, alkaloids and phenolic compounds (Krishnaiah *et al.* 2007). Terpenoids and phenols exhibit various important pharmacological activities like anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities (Mahato and Sen 1997). Terpenoids are very important in attracting useful mites and consume the herbivorous insects (Kappers *et al.* 2005). Alkaloids are used as anesthetic agents and are found in medicinal plants (Herourat *et al.*, 1988; Kumbhar and Godghate, 2015).

Ajuga bracteosa Wall ex. Benth. of family Lamiaceae is commonly known as 'Bungle' in English and 'Jan-i-adam' in Kashmiri. It is a perennial erect, ascending hairy herb, often prostrate with oblanceolate or sub-spathulate leaves and grows up to 5-50 cm tall. It is distributed in subtropical and temperate regions Bhutan, Pakistan, Afghanistan, China, Malaysia at an altitude of 1300 m asl. In India, it abounds in western Himalaya, plains of

Punjab, upper Gangetic plains of India (Khare, 2007) and in Kashmir at an altitude of 1300 m (Chandel and Bagai 1999). It is found along roadsides, open slopes, and rock cervices up to 1500 m above mean sea level (Chauhan 1999; Upadhyay *et al.* 2011). In Pakistan it is found in northern hilly areas, where in local Hindi/Punjabi language it is called kori booti (means bitter herb) owing to its bitter taste. It is found along roadsides, open slopes, and rock cervices. The plant is used for the treatment of gout, rheumatism, palsy and amenorrhoea. Locally the leaves help in curing headache, pimples, measles, stomach acidity, burns, boils. It is effectively used against jaundice, hypertension, sore throat and as a blood purifier.

Materials and Methods

Collection and air drying of plant material: Aerial parts of *Ajuga bracteosa* were collected from Sinthan Top area of District Anantnag (Kashmir) in the month July, 2013. The plant was identified at the Centre of Biodiversity and Plant Taxonomy, Department of Botany, University of Kashmir, Srinagar, J and K and a voucher specimen (JKASH/CBT/226; Dated 08. 08. 2014) was deposited there. The parts were allowed to dry under shade (30 °C) for 8-10 days.

Preparation of extracts: After shade drying, the aerial parts were macerated to fine powder, 1 kg of leaves were extracted successively with hexane for defatenning and methanol for 16 h using Soxhlet apparatus. The extracts were filtered through a Buchner funnel using Whatman No. 1 filter paper, and all the extracts were concentrated to dryness under vacuum using a Heidolph rotary evaporator, yielding hexane, ethyl acetate, methanol and aqueous crude extracts of 65, 52, 46 and 36 g respectively. All the extracts were stored at 4°C in air tight glass bottles before use.

Phytochemical screening: Chemical tests were carried out on the extracts using standard procedures to identify the constituents (Harborne 1984; Evans 1989; Sofowora, 1993; Okwu 2004).

Test for tannin: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue- black colouration.

Test for saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion.

Test for flavonoids: Three methods were used to determine the presence of flavonoids in the plant sample. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids.

A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for steroids: Two ml of acetic anhydride was added to 0.5 g extract of each sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids (Salkowski test): Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoid.

Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) **Mayer's Test:** Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
b) **Wagner's Test:** Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
c) **Dragendroff's Test:** Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.
d) **Hager's Test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a. **Molisch's Test:** Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.
b. **Benedict's Test:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.
c. **Fehling's Test:** Filtrates were hydrolysed with dilute HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of Phytosterols

a. **Salkowski's Test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.
b. **Libermann Burchard's test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of phenols: Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of proteins and amino acids

a. **Xanthoproteic Test:** The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.
b. **Ninhydrin Test:** To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Results and Discussion

Therapeutic values of medicinal and aromatic plants (MAPs) are due to the presence of major bioactive constituents like alkaloids, phenolics, flavonoids, tannins, cardiac glycosides, terpenes, saponins, steroids etc. The phytochemical investigation of *Ajuga bracteosa* extracts in the present study revealed presence of different active ingredients (secondary plant metabolites) like flavonoids, phenolics, alkaloids, tannins, cardiac glycosides, terpenes, saponins, steroids, carbohydrates, amino acids and proteins as shown in Table 1. It supports the resourcefulness of the plant extract (Sofowora, 1993).

Table 1: Qualitative phytochemical screening of *Ajuga bracteosa*

Phytoconstituents	Test	Result
Alkaloids	Wagner's test	+ +
Phenolics	phenol test	+ +
Tannins	Ferric chloride test	+ +
Cardiac glycosides	Keller-Killani test	+ +
Terpenes	Salkwaski's test	+
Flavonoids	Shinoda's test	+ +
Saponins	Frothing test	+
Steroids	Libermann-Buchard's test	+
Carbohydrates	Molish test	+ +
Proteins	Biuret test	+
Polysterols	Salkowski's Test	+
Amino acids	Ninhydrin Test	+

(++) = strong presence, (+) = moderate presence

From ancient times, medicinal plants are being used as remedies for various diseases in human. In today's industrialized society, the use of medicinal plants has been traced to the extraction and development of several drugs as they were used traditionally in folk medicine (Shrikumar and Ravi 2007). Medicinal plants have potent phytoconstituents which are important source of antibiotic compounds and are responsible for the therapeutic properties (Jeeva *et al.* 2011; Jeeva and Johnson 2012; Florence *et al.* 2012, 2014; Joselin *et al.* 2012, 2013; Sainkhediya and Ray 2012; Sumath *et al.* 2014). These phytoconstituents endow them with medicinal properties. Many plants possess antioxidant properties because of the presence of phenolic compounds (Brown and Rice-Evans 1998; Krings and Berger 2001). These phenolic compounds possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al.* 2007). Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie 1996). They also are effective antioxidant and show strong anticancer activities (Salah *et al.* 1995; Del-Rio *et al.* 1997).

Besides, most of the phytochemicals are known to have therapeutic properties such as insecticidals (Kambu *et al.* 1982), antibacterial, antifungal (Lemos *et al.* 1990) and anticonstipative (Ferdous *et al.* 1992) activities etc. The plants thus find their medicinal values due to the presence of these phytochemical constituents. The presence of various phytochemicals in the tested plant reveals that this plant may be a good source for production of new drugs for various ailments.

Ajuga bracteosa also contain saponins which are known to produce inhibitory effect on inflammation (Just *et al.*, 1998). Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al.*, 2000). Steroids, another important phytoconstituent present in Ajuga bracteosa, have been reported to possess antibacterial properties (Raquel 2007) and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001). Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Nobori *et al.* 1994). It has been reported that alkaloids possess analgesic (Antherden, 1969), antispasmodic and antibacterial (Stray, 1998; Okwu and Okwu, 2004) properties. Glycosides are known to lower the blood pressure according to

many reports (Nyarko and Addy 1990). Thus from the present study, it could be suggested that the identified phytoconstituents from *Ajuga bracteosa* make the plant valuable for bioactive compounds of sustainable medicine.

Conclusion

The medicinal plants are the source of the secondary metabolites i.e., alkaloids, flavonoids, terpenoids, phlobatannins and reducing sugars. Medicinal plants play a vital role in preventing various diseases. The antidiuretic, anti-inflammatory, antianalgesic, anticancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. Thus, *Ajuga bracteosa* can be used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. Thus we hope that the important phytochemical properties identified in this study in the local plant of Kashmir Himalaya will be helpful in copping different diseases of this particular region.

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Smoking Habits and their Effects on Different Histological Types of Non-Small Cell Lung Cancer (NSCLC) among Kashmiri Population

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Abstract
This prospective study was conducted with the aim to determine the effect of smoking habits on different histological types of non-small cell lung cancer among male and female Kashmiri population. A total of 65 patients out of which 55 males and 10 females with histological documentation of non-small cell lung cancer were enrolled in the study. Patients with malignancy were presented with average age of 58.94 years; most of them were within the age range of 50 to 84 years. A detailed history was taken in each case that revealed active smokers 80% of which cigarette smokers were 59.61%, hookah (water pipe) 32.69 % and bidi smokers 13.46 %. It was also observed that non-small cell lung cancer was more common among cigarette smoker (80%) followed by active hookah smokers (5%). Adenocarcinoma was the main histological type of lung cancer associated with all types of smoking habits, followed by squamous cell carcinoma. The major symptom which was seen in the patients having non-small cell lung cancer is cough (53 out of 65). Beside cough other major symptoms were dyspnea 56.92 % and weight loss 56.92%. Haemoptysis were seen in 33.8% of the patients, 30.7% patients were having chest pain, 29.23 % patients were having fever and 29.23 % patients complain of having hoarseness of voice.
Keywords: Smoking, non-small cell lung cancer, adenocarcinoma, Kashmiri population

Introduction

Lung cancer is the most common cancer worldwide accounting for about 18% of all cancers in men (Jemal and Bray, 2011; Brambilla *et al.*, 2001). There are 2- 2.5 million cancer cases present at any given point of time in India (Rwat *et al.*, 2009). Lung is the leading site of cancer in males as per the three urban cancer registries of India (Ganesh *et al.*, 2011.). The incidence of lung cancer is increasing rapidly, mainly due to progressive change in life style (Dhar *et al.*, 1993; Parkin., 1989). It remains a major health problem in Kashmir valley and constitutes nearly 9.9% of all cancers (Shah *et al.*, 1990; Koul *et al.*, 2010). The epidemiology of lung cancer is dominated by its association with smoking. The dramatic increase in cancer death rates among men and the more recent increase among women can be attributed to increase in cigarette consumption (Forbes *et al.*, 2006). Lung cancer is responsible for about one million deaths per year at present and will rise to three million per year by the year 2010 (Long., 2012). Smoking is a major risk factor for lung cancer with approximately 90% to 95% of new lung cancers resulting from active smoking (Ferlay *et al.*, 2015). Tobacco exposure has been strongly associated with non-small cell lung cancer than any other type (Riely *et al.*, 2008). The current study was undertaken to determine the risk of smoking habits on different histological types of lung cancer in Kashmiri population.

Material and Methods

A total of 65 patients with the diagnosis of non-small lung cancer were studied prospectively between January 2014 and June 2015 in the Department of Clinical Biochemistry at SKIMS Srinagar, Kashmir. All patients had

histologically and cytologically proven cancer of the lung determined through CT guided FNAC/ biopsy 80%, bronchoscopy 15.38% and both 4.61%. Besides routine history, a detailed history was taken in each case regarding smoking habits that included duration of smoking and the type of smoking. The occupational history and the association with non-small cell lung cancer were also stressed upon in the history.

Results and Discussion

With effect from January 2014 to June 2015, 65 patients with histologically proven non-small cell lung cancer were enrolled in the study. There were 55 male and 10 female patients. Majority of patients were in the age group of 50-84 years. All patients had histologically /cytologically proven cancer of the lung determined through CT guided FNAC/ biopsy 80%, bronchoscopy 15.38% and both 4.61 % (Figure 1). As per the occupational status, 34% were farmers, 27.69% service class, 18.46% housewives and others 23.07% (Table 1). The history of active smoking was present in 80% and 20% were non-smokers but had definite history of house hold smoke exposure since their childhood/adolescence. Among 80% of active smokers, 59.61% were cigarette smokers. Hooka and Bidi smokers were 32.69% and 13.46% respectively (Table 2). It is interesting to note that 45.6% of patients had started smoking below 20 years of age. Majority, 64.61% had smoked for 11-30 years. Adenocarcinoma was the main histological type of non-small cell lung cancer associated with all types of smoking habits, followed by squamous cell carcinoma. The major symptom which was seen in the patients having non-small cell lung cancer is cough (53 out of 65). Beside cough other major symptoms were 56.92 % dyspnea and 56.92% weight loss. Haemoptysis were seen in 33.8% of the patients, 30.7% patients were having chest pain, 29.23 % patients were having fever and 29.23 % patients complain of having hoarseness of voice (Figure 2).

Table 1: Occupational status of patients with NSCLC

Occupation	Number
Farmer	20
Service Class	18
House Wife	12
Others	15

Table 2: Smoking status of patients diagnosed as NSCLC.

Smoking status	Number	Percentage
Smokers	52	80
Cigarette	31	59.61
Hookah (water pipe)	17	32.69
Bidi	7	13.46
Non Smokers	13	20

Lung cancer is the most frequently malignant disease and the most common cause of cancer death in the world. A recent study shows that Srinagar, Jammu and Kashmir has the highest incidence of lung cancer among males in India (Koul *et al.*, 2010). The lung cancer was predominantly seen in male, who accounted for 84.61%. The male female ratio was 1:1 in this study. A significant proportion of the cases in the study were within range of 50-70 years (68%) the mean age was 58.94 years. Most common symptom experienced by the patients was cough and were associated with 53 patients who were suspected for lung cancer. The next most common symptom reported were 56.92% dyspnea and 56.92% weight loss. Haemoptysis were seen in 33.8% of the patients, 30.7% patients were having chest pain, 29.23% patients were having fever and 29.23% patients complain of having hoarseness of voice (Figure 2). Most common radiological finding in lung cancer patients was space occupying lesion (mass) which was found in 66.15% of all malignant patients (43). It was on the right side of lung in 32 patients (74.41%) and on the left side of lung in remaining 11 patients (25.58%). Other major radiological finding was pleural effusion in 22 patients (33.84%). Finally out of all NSCLC cases fifteen i.e 23.07% patients had squamous cell

carcinoma, fifty i.e 76.92% had adenocarcinoma. The most histological type among smokers (cigarette, bidi and hookah) was adenocarcinoma of about 80.76% (Figure 3).

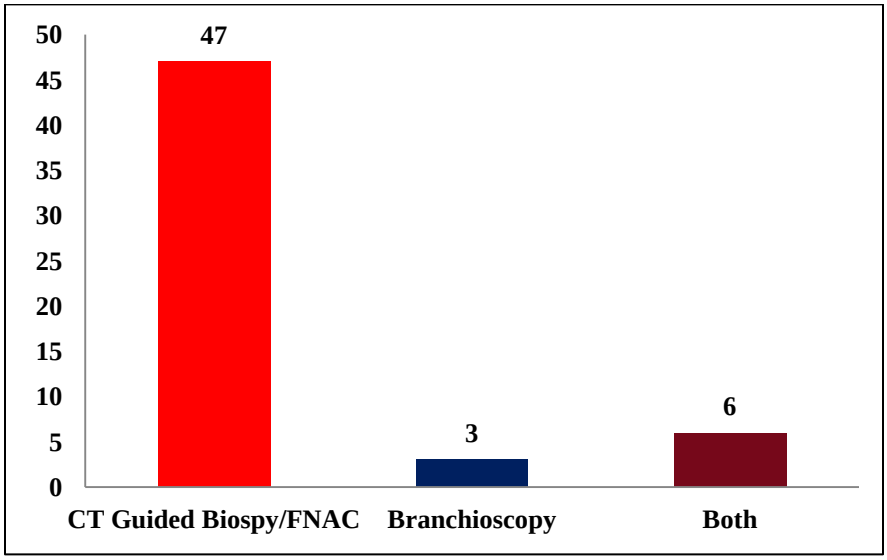

Figure 1: Diagnostic procedures used for identification of NSCLC patients (enrolled in the study)

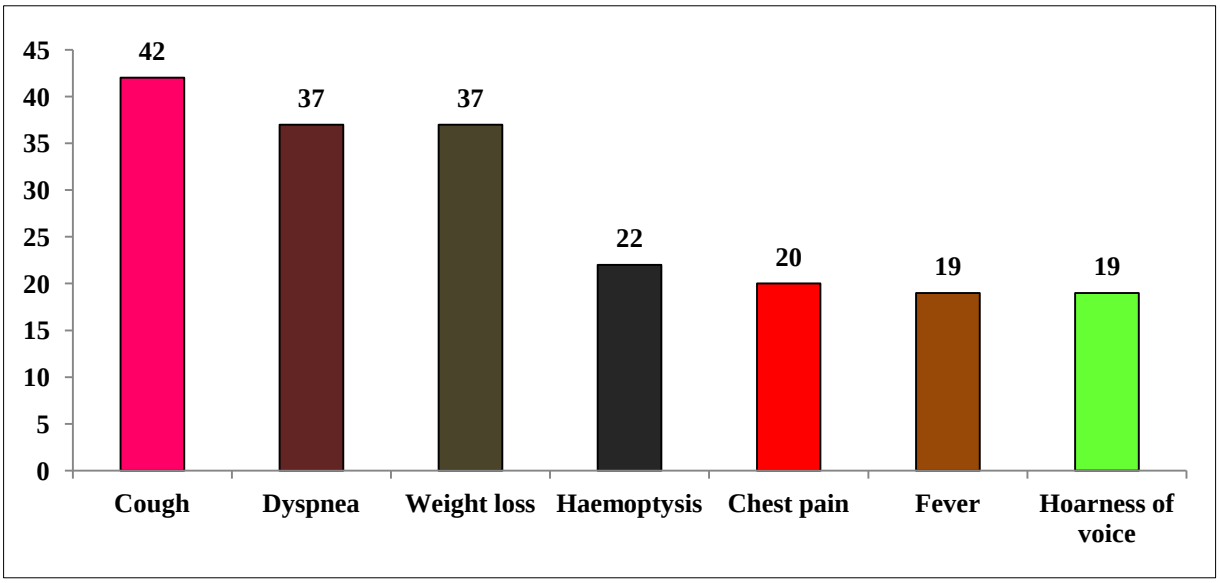

Figure 2: Symptoms found in NSCLC patients (enrolled in the study)

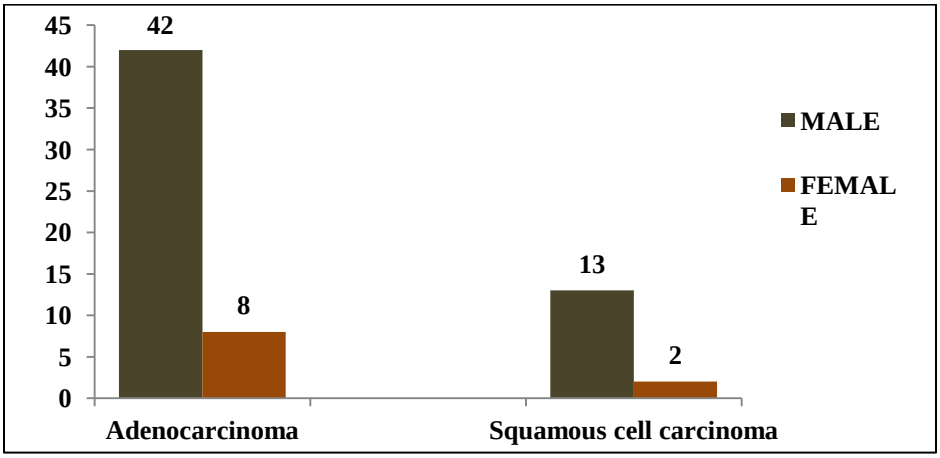

Figure 3: Histological type found in the patients having NSCLC

Conclusion

The present study demonstrates that majority of the patients having adenocarcinoma were males and smokers. Lung cancer was more common among farmers, who were active hooka smokers. The findings of the current study are limited due to small sample size in the strata, our results need to validate by large size studies.

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Elemental Analysis of Mushroom Flora from Budgam District of Kashmir , India

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Abstract
Three mushroom species *Ganoderma applanatum, Fomes fomentarius* and *Pleurotus ostreatus* from two different sites of the Budgam district were analyzed for the variation in elemental composition. Much variation was not found in the macronutrient content of N, H, S, C and crude protein in the samples from two sites. There was a significant variation in the concentration of micronutrients such as; Cu, Zn, Fe, Mn, Cd and Cr.
Keywords: Trace metals, mushrooms, atomic absorption spectrometry.

Introduction

Mushrooms are mycorrhizal, parasites and saprophyte. They include members from both basidiomycota and ascomycota. Mushrooms have been popular food supplement in many countries and are being cultivated artificially world wide for their edibility and delicacy. Wild growing edible mushroom collection has become a hobby in many cultures for instance, Czech Republic 72% of families collect mushrooms with a mean yearly level of 7 kg per household (Kalac and Svoboda 2000). They fall between the best vegetables and animal protein source. Mushrooms are valuable health foods, low in calories, high in vegetable proteins, iron, zinc, chitin, fibre, vitamins, amino acids and minerals. They also have a long history of use in traditional Chinese medicine (Demirbas, 2001; Mendil *et al.* 2004; Racz *et al.* 1996). Mushrooms are rich sources of essential amino acids, water-soluble vitamins (riboflavin, biotin and thiamine) and essential minerals (Chang and Buswell, 1996; Buigut, 2002). In general, their fruiting bodies on dry weight basis contain about 39.9% carbohydrate 17.5% protein and 2.9% fats, with the rest constituted of minerals (Demirbas, 2001; Latiff *et al.* 1996; Mendil *et al.* 2004). It has been reported recently that compared to plants certain metals like Cd, Hg, Pb, As, Cu, Ni, Ag, Cr, Hg accumulate in fungal fruiting bodies (Malivewska *et al.* 2004; Meistrik and Lepsova, 1993; Schimitt and Sticher, 1991; Wondratschek and Roder, 1993), consequently effort has been made to evaluate the possible hazardous effects to human health from the ingestion of mushrooms (Gast *et al.* 1988).

The essential metals can also produce toxic effects when the metal intake is excessively elevated. Recently, studies have drawn attention to the metal pollution of soil and plant samples (Tuzen, 2003). The contents of metals are related to species of mushroom, collecting site of the sample, age of fruiting bodies and mycelium and distance from the site that is polluted. Metals such as Zn and Se are essential metals since they play important role in biological systems, where as Cd ad Cr are non- essential metals as they are toxic, even in traces (Schroeden, 1973). The concentration of 4 metals in samples of mushroom fruiting bodies representing three species, one edible fleshy fungi and two inedible bracket fungi, has been determined by atomic absorption spectroscopy.

Kashmir is located between Jammu and Ladakh region of JandK state in India. Complex geography and vegetation as well as diverse climatic conditions provide a variety of natural habitats for a rich resource of mushrooms. The seasons are normally wet with mild temperatures; especially spring and autumn are suitable for mushroom growth. People who live in Kashmir widely consume wild edible mushrooms because of their delicacy and abundance. Kashmir is important exporter of wild mushrooms like *Morchella* and *Pleurotus* hence has a large edible mushroom potential. Studies on the mushroom samples for the levels of metals determination are scarce or equal to nothing in Kashmir. In this study, the

levels of metals in mushroom samples collected from Rawalpora, Kashmir were determined by graphite furnace atomic absorption spectroscopy (GFAAS) after acid digestion and by elementar.

Material and Methods

Three different fully matured mushroom species were collected from two different sites in Rawalpora area of Kashmir region during August 2005. Samples were uprooted from its substratum with the aid of a scalpel and after complete cleaning samples were oven dried at 40–105^{o}C for 2-24 hrs. Dried samples were homogenized and stored in polyethylene Ziplock bags prior to analysis. The samples of mushroom include edible fleshy fungi *Pleurotus ostreatus* and inedible, hard fungi *Fomes fomentarius* and *Ganoderma appanatum*. All the plastic ware and glassware were cleaned by soaking overnight in a 10% nitric acid solution and then rinsed with deionized water.

Sample preparation and analysis by GFAAS: 0.5g of the dried and homogenized samples of mushrooms was taken and was digested in a mixture of concentrated acids (2:1) HNO_3: $HClO_4$ in the 50 ml digestion tubes over a block digestor. Small pyrex funnels were placed over the tubes and the samples were heated to 60^{0}C for 15 min, further the temperature was increased to 120^{0}C and the digestion was carried out for 75 min until the samples cleared. Afterwards the samples were cooled down and the volume was made upto 50ml with milli Q water containing 2% HNO_3 (Gupta, 1999).

Metal analysis was done through Atomic absorption spectrophotometer for Cr and Cd (Graphite furnace based model Analytic Xena Zeenit 65) according to the protocol prescribed in the manual of the apparatus. The analysis was performed in Central Instrumentation Facility (CIF), Jamia Hamdard, New Delhi. The analysis of Cu, Fe, Mn, Zn was performed in the Pomology Division, SKUAST-K, Shalimar, using atomic absorption spectrophotometer (AAS 4141). The samples were analyzed in triplicates along with sample blanks. The standard curve for each metal was analyzed utilizing analytical grade standard metal solutions (Merck Chemical Company).

Sample packing and analysis by CHNS analyzer: 10 mg of sample was packed in aluminum packs along with spatula of tungsten oxide and wolfram (VI) oxide mixture (Merck) and loaded. Analysis of carbon, hydrogen, nitrogen, sulphur and protein percentage where done through Elemental Analyzer System GmbH (Model VarioEL III) according to the protocols prescribed in the manual of the machine.

Results and Discussion

The habitat, family, edibility and medicinal activity of mushroom species are shown in Table 1. Analysis was carried in three different mushrooms species viz. *Fomes fomentarius, Ganoderma applanatum*, *Pleurotus ostreatus* from two different sites of Budgam district. They were selected based on their availability at the time of analysis. Total content of nitrogen, hydrogen, carbon, sulphur and crude protein are shown in Table 2. All the chemical concentration was determined on a dry weight basis.

Iron content ranged from 96.4 µg g^{-1}dw to 729.5µg g^{-1}dw for the site I and from 252.7µg g^{-1}dw to 632.2µg g^{-1}dw for site II. The highest concentration of Fe was found in *Pleurotus ostreatus* with 729.5µg g^{-1}dw (Site I) and *Ganoderma applanitum* with 632.2µg g^{-1}dw (Site II). Iron values for various mushrooms have been reported to be in the ranges: 31.3–1190 µg g^{-1}dw (Sesli and Tuzen, 1999), 568–3904 µg g^{-1}dw (Turkekul *et al.* 2004) and 56.1–7162 µg g^{-1}dw (Isılogglu *et al.,* 2001), respectively. Iron values for the species investigated presently are in agreement with those reported in the literature. The minimum and maximum concentration of Manganese (Mn) in collected samples ranged from 11.4µg g^{-1}dw to 17.6µg g^{-1}dw for site I and 1.7µg g^{-1}dw to 73.2µg g^{-1}dw for site II respectively. The highest concentration of Mn i.e. 73.2µg g^{-1}dw for site II and 17.1µg g^{-1}dw for site I was recorded in *Pleurotus ostreatus*. The manganese values recorded so far in the mushrooms are 7.6–56.2 µg g^{-1}dw (Demirbas, 2001), 21.7–74.3µg g^{-1}dw (Isildak *et al*, 2004) and 7.1–81.3 µg g^{-1}dw (Tuzen, 2003), respectively. Mn contents obtained in this study are in accordance with literature. Zinc (Zn) was detected in all mushroom samples, which range from 42.3µg g^{-1}dw to 76µg g^{-1}dw for site I and 70.6µg g^{-1}dw to 115.5µg g^{-1}dw for site II. The highest Zn content (115.5µg g^{-1}dw) was recorded in

Fomes fomentarius for site II. Zinc is widespread among living organisms due to its biological significance. Mushrooms are known as zinc accumulators in the fruiting body (Bano *et al.* 1981; Isılogglu *et al.* 2001). Zinc concentrations of mushroom samples in the literature have been reported to be in the ranges: 40.3–64.48 µg g^{-1}dw (Mendil *et al.* 2004) and 29.3–158 8 µg g^{-1}dw (Isılogglu *et al.* 2001). The results obtained in the present study are more or less similar to the earlier studies (Figure 1).

Table 1: Edibility and medicinal activity of three mushroom samples collected from two different sites in Budgam district of Kashmir

S. No.	Mushroom species	Family	Habitat	Edibility	Medicinal Activity
1.	*Fomes fomentarius*	Polyporaceae	Both on the stumps of cut trees and tree	In-edible	Antiinflammatory
2.	*Ganoderma applanatum*	Ganodermataceae	Grows at the base of *Salix* tree	In-edible	Anti-bacterial, anti-HIV, Immuno-modulatory
3.	*Pleurotus ostreatus*	Pleurotaceae	Populus tree and *Salix* stump	Edible	Immuno-Modulatory, Anti-tumor, anaemia

Table 2: Variation of N, H, C, S and Crude protein content in three mushroom samples collected from two different sites in Budgam district of Kashmir

Mushroom Species	Site	N content %	H content %	C content %	S content %	Crude protein content %
Fomes fomentarius	S1	4.862	6.304	39.73	ND	21.30
	S2	4.048	6.818	42.15	ND	17.73
Ganoderma applanitum	S1	4.424	4.248	26.70	ND	19.38
	S2	9.892	6.481	43.07	ND	43.33
Pleurotus ostreatus	S1	7.370	7.439	40.08	0.073	32.28
	S2	4.738	7.223	37.71	0.022	20.75

Contents are given as the arithmetical mean of three independent replications.

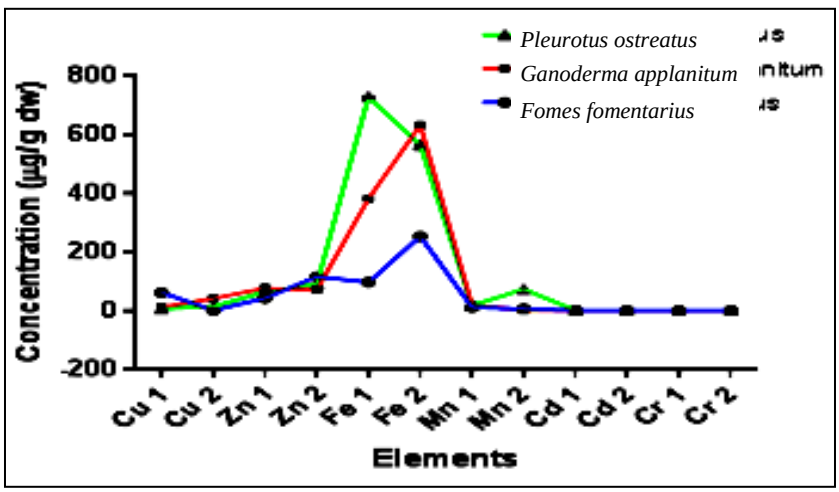

Figure 1: Variation of metal content in three mushroom samples from two different sites in Budgam district of Kashmir

Copper concentration was between 6.3 µg g^{-1}dw to 61.4 µg g^{-1}dw for site I and 0.4µg g^{-1}dw to 41.5µg g^{-1}dw at site II; the highest concentration 41.5µg g^{-1}dw was recorded in *Ganoderma applanatum* at site II. Very least amount of copper (0.4µg g^{-1}dw) was detected in *Fomes fomentarius* at site II. Copper contents of mushroom samples in the literature have been reported to be in the ranges: 4.71–51.0µg g^{-1}dw (Tuzen, *et al.* 1998), 12–181µg g^{-1}dw (Tuzen *et al.* 2003) and 10.3–145µg g^{-1}dw (Sesli and Tuzen, 1999), respectively. Other studies also report copper from different mushrooms in the range of 34.5–83.0µg g^{-1}dw (Demirbas, 2002), 10.0–14.0 µg g^{-1}dw (Isılogglu *et al.* 2001) and 21.1–42.6µg g^{-1}dw (Sivrikaya *et al.* 2002), respectively. Copper is one of the essential minerals that help iron in making red blood cells and delivering oxygen to every part of the body. Chromium observed ranged from 0.049µg g^{-1}dw to 0.079µg/g dw for site I and 0.049 µg/g dw to 0.136µg/g dw for site II. Chromium values in mushroom samples have been earlier reported to be in the ranges: 0.16–4.86 µg g^{-1}dw (Malinowska *et al.* 2004), 0.87–2.66µg g^{-1}dw (Tuzen, 2003) and 7.0–11.0 µg g^{-1}dw (Siverikaya *et al.* 2002), respectively. The chromium levels in mushrooms analysed for this study were found to be lower than those reported in the literature. Cadmium (Cd) concentrations in mushroom species ranged from 0.127µg g^{-1}dw to 0.332µg g^{-1}dw at site I and 0.041µg g^{-1}dw to 0.652 µg g^{-1}dw at site II. The highest concentration of cadmium was found in *Ganoderma applanatum* (0.652 µg g^{-1}dw at site II). Cadmium contents of mushroom samples in the literature have been reported to be in the ranges: 0.81–7.50µg g^{-1}dw (Svoboda *et al.* 2000), 0.10–0.71 µg g^{-1}dw (Mendil *et al.* 2004), 0.28–1.6µg g^{-1}dw (Mendil *et al.* 2004) and 0.12–2.60µg g^{-1}dw (Malinowska *et al.* 2004). Our cadmium levels were found in accord with the results reported in the literature; however, value recorded for some species in the present study is much lower than those reported earlier in the literature. The concentration of nitrogen, hydrogen and carbon ranged from 4.048% to 9.892%, 4.248% to 7.439%, 26.70% to 43.07%, across all the mushrooms analyzed from site I and site II respectively. Only *Pleurotus ostreatus* contained Sulphur that too very meager amount i.e. 0.022% and 0.073% from site I and site II respectively. The low concentration of sulphur in mushrooms is in accordance with the observations made for analysis of sulphur containing amino acids by different scientist. Most of the species of mushrooms are deficient in sulphur containing amino acids. The crude protein content ranged from 17.73% to 43.33% from mushrooms in site I and site II respectively. Now- a-days mushroom is considered as the corner stones of health care system due to presence of many helpful phytochemicals in alleviating some serious diseases. In modern system of disease control, mushrooms containing strong antioxidants properties or phytochemicals neutralize the injurious effects of free radicals as scavengers and thus help in specific body functions in reducing the risk of incidence of many diseases like cardiovascular problems, various types of arthritis, cancer, AIDS and various other degenerative diseases. The predominant mushrooms showing promise for their antiviral and other medicinal activities are polypores- the so-called bracket fungi or woody conks like species belonging to Genus *Ganoderma, Fomes* and *Trametes* (Collins and Ng, 1997; Hattori *et al.* 2011). The current study focuses to mushrooms especially those belonging to *Polyporaceae*, as a rich frontier of new medicines. Many of these are long term residents of nutrient recycling by decomposing aged trees. In a time when new antiviral medicines are critically needed, mushrooms stand out as an untapped resource and deserve intensive studies.

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Effect of Mughal Road on Land Use / Land Cover of Sukh Saria-A Catchment Area of Rambiara Nallah, Shopian

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Abstract
The present study was carried out to analyze the effect of Mughal road on dynamics of land use/land cover using geospatial techniques of remote sensing and GIS on Sukh Saria- a catchment area of Rambiara Nallah in Hirpora of Shopian Distract, Kashmir. During the 20 years time period, an unprecedented decrease in dense forest area has caused a key land use change which has occurred due to construction of the famous Mughal road. This has led to increasing emissions of CO_2 into the atmosphere which in turn leads to climate change. However, the challenges of climate change can be effectively overcome by terrestrial carbon sequestration.
Keywords: Land use, land cover, remote sensing, catchment, carbon sequestration

Introduction

Land is the most important natural resource which embodies soil, water and associated flora and fauna involving the total ecosystem. Land cover refers to physical and biological cover over the surface of land, including water, vegetation, bare soil, and/ or artificial structures (believes land use involves both the manner in which the biophysical attributes of the land are manipulated and the intent underlying that manipulation-the purpose for which the land is used (Ellis and Pontius Jr., 2006. Turner *et al.* (1995). Land cover is also used to describe different natural habitats, deserts, forests, woodlands, glaciers and water bodies as well as habitats manipulated by man. Though humans have been modifying land to obtain food and other essentials for thousands of years, current rates extends and intensities of land use and land cover change are far greater than ever in history, driving unprecedented changes in ecosystem and environmental modification at local, regional and global scales (Xieo *et al.*, 2006). These changes encompass the greatest environmental concerns of human populations today, including climate change, biodiversity loss and the pollution of water, soil and air. Therefore, land use and land cover are two essentials unfolding the terrestrial environment in connection with both natural as well as anthropogenic activities (Bender *et al.*, 2005, Mendoza *et al.*, 2010).

Forest roads are the most costly structures in forestry. Inefficiently constructed forest roads can cause severe environmental impacts including road surface erosion and sediment yield (Fu *et al.*, 2010), pollution of waters, direct loss of habitat by conversion of artificial land cover into an artificial surface (Geneletti D., 2003) and indirect loss of habitat by the fragmentation of an ecosystem into smaller and more isolated patches (Chomitz *et al.*, 1996). Large areas of forest are destroyed during road construction which not only results in economic losses, but changes the conditions of the environment (Jadczyk, 2009).Road effects take place in the contexts of environmental settings, their history, and the state of engineering practices, and must be evaluated in those contexts for best management approaches.

Remote sensing plays an important role in generating information about the latest land use land cover pattern in an area and its temporal changes through times. The information being in digital form can be brought under geographical information system (GIS) to provide suitable platform for data analysis, update and retrieval. The study focuses on the effectiveness of satellite data for land use/land cover change of the study area due to the construction of a famous mughal road.

Study Area

The study area lies at an altitude of 2546 m above the mean sea level within geographical coordinates of 33° 39'55"N and 74° 39' 40"E (Figure 1). The study area is located at the bank of Rambiara Nallah, within the heart of Hirpora wildlife sanctuary. The climatic conditions of the study area are somehow different from Kashmir valley. The temperature is in between 6°C to 27°C in summers and the winters are chilly with heavy snowfall. Sukh Sarai is very beautiful in its natural beauty, which comprises of western mixed coniferous forests and deciduous sub-alpines scrub forests, also the presence of pastures and meadows beautify the area and provide a grazing site during summers.

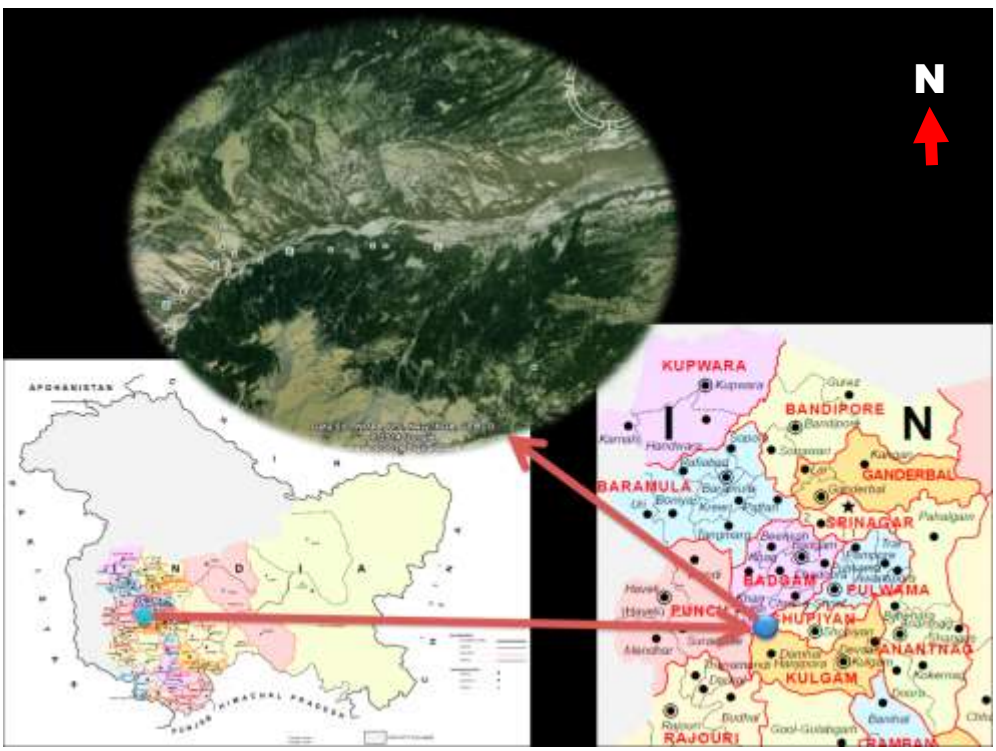

Figure 1: Location map of study area

Materials and Methods

IRS-IC LISS III with 23.5 meter resolution acquired on 25 October 2010 and Landsat Thematic Mapper data of 25 October 1990 were used as source data. The scheme adopted for land use/ land cover classification is the level I and II of NRSA with local modification. The area of Sukh Sarai were accordingly divided into six classes namely, dense forest, sparse forest, scrub land, pasture land, waste land, and water bodies. The remotely sensed data was geometrically corrected using toposheets as references. On screen digitization approach was used. During the

computation of change detection of area under Land use/ land cover categories, the percentage change of the total area was calculated , which is the change of area in a particular category divided by total area of catchment multiplied by 100.

Results and Discussion

In the present study, the Study area was classified using on screen digitization technique into 06 land use/ land cover classes. The composition and distribution of land use/land cover types of images included: dense forest, sparse forest, scrub land, pasture land, waste land, and water bodies. The land use/ land cover map shows the spatial and temporal variation in the area (Table 1; Figure 2 and 3).

Table 1: Land use/ Land cover data of study area as depicted/ extracted from satellite images.

S. No	Landuse/ Land cover classes	Area in 1990 (Hectares)	Percentage	Area in 2010 (Hectares)	Percentage	Percentage change
01	Dense forest	96	30.57%	**56**	17.82%	**-12.73**
02	Sparse forest	**138**	43.94%	**46**	14.64%	**-29.29**
03	Scrub land	45	14.33%	**136**	43.31%	**28.98**
04	Pasture land	24	7.64%	65	20.70%	13.05
05	Waste land	**0**	0%	6	1.91%	1.91
06	Water bodies\	11	3.50%	**5**	**1.59%**	**-1.91**

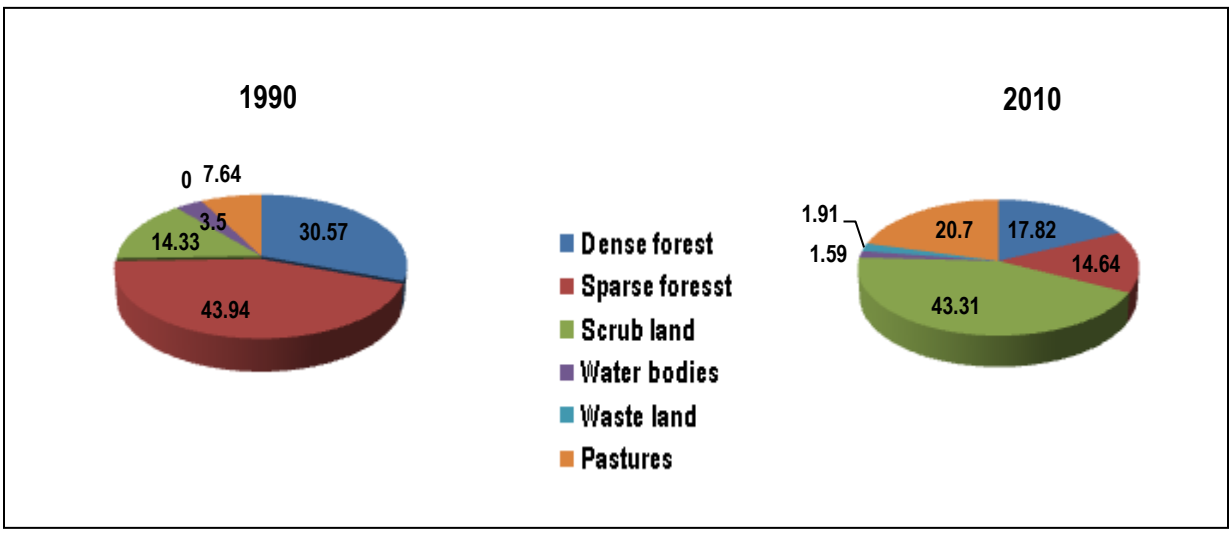

Figure 2: Land use/land covers percentage change

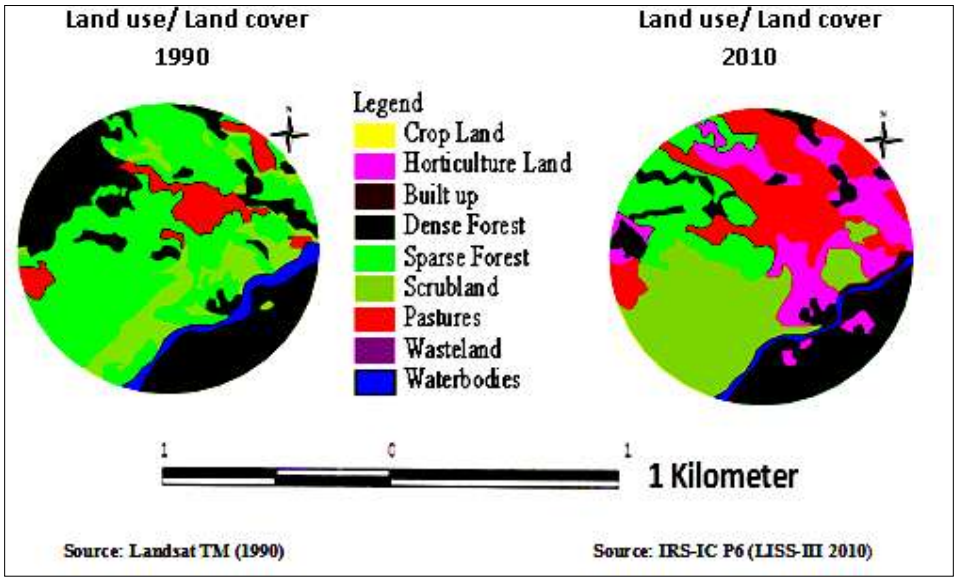

Figure 3: Satellite images shows land use/land cover maps

The present study reveals that scrub land has shown a noticeable increase from 14.33% in 1990 to 43.31% in 2010 respectively, which contributes about an increase of 28.98% from last two decades. This increase may be at the cost of decrease of forest cover due to construction of Mughal road, tourism. These findings are in agreement with the findings of (Gunilla *et al.*, 2000). During study it has been found that the largest pasture land is present which contributes about 20.70 percent of the total area, which acts as a grazing site in summers as the tribal's and nomads take their livestock to these areas because of pleasing temperature. This increase in pasture land is mainly due to the conversion of grass land, woodland and forest into pastures. These findings are in agreement with findings of (Houghton, 1994; Williams, 1994). Waste lands were absent before the construction of mughal road was 0 ha in 1990 and 6 ha in 2010.This increase is due to the conversion of forest land into waste land by the way of deforestation. These findings are in collaboration with the findings of (Kaul *et al.*, 2009). Dense forests have also shown a decreasing trend of about 17.82 percent of the total area has shown in fig.2.This decrease in the forest area is mainly due to natural factors, population pressure and increasing demands of people for timber, fuel wood, land for settlement, roads (Mughal road), agriculture etc have lead to declining of the forest area. These findings are in alliance with the findings of Sparse forests have shown a decreasing mode 43.94 percent in 1990 to 14.64 percent(Sen, 2002; Sharma and Roy, 2007; Farooq and Rashid, 2010). This decrease in sparse forest is mainly due to population expansion, increasing demand of the people for firewood, fuel wood and other minor forest products. These findings are in conformity with findings of Nussar, 2000 and Sen, 2002. Lack of employment opportunities also compels the people to depend on forests (Sharma *et al.*, 2009). Water bodies have also shown an alarming decrease from 11 ha in 1990 to 5 ha in 2010 respectively. This decreasing area of the water bodies may be due to land transformations. These findings are in compliance with the findings of Fazal and Amin, 2011.

Conclusion

The key finding of the present study includes the immense degradation of dense forests, decrease in water bodies, increase in pasture land and scrub land. These changes have clearly depicted that the negative impacts of mughal road construction along the catchment of Rambiara Nallah- a forest area. Therefore, forest road managers should

consider not only the total road cost but also environmental impacts i.e. climate change, biodiversity loss, soil degradation, water pollution caused by road construction and use. However, carbon sequestration acts as a one of the effective tool for management of forest ecosystem. Therefore, carbon sequestration is truly a win-win strategy in this regard. Thus, it becomes imperative that the government should prepare working plans for the effective management.

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Extreme Adaptations of Extremophiles: Extremozymes

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Abstract
Extremozymes are the enzymes that are derived from extremophiles, microorganisms that thrive well inextreme environments such as extremes of salinity, acidity, alkalinity, temperature or pressure as well as other seemingly drastic surroundings. Extremozymes provide new possibilities for biocatalysis and biotransformation due to their extreme thermostability.Extremozymessuch as cellulases, amylases, xylanases, proteases, pectinases, keratinases, lipases, esterases, catalases, peroxidases and phytases have useful applications in molecular biology, medical research, industrial food or feed technology, detergents and cosmetics. These enzymes are highly stable at extreme conditions.
Keywords: Extremophiles, extremozymes, enzymes.

Introduction

Extremophiles are the organisms which permanently experience environmental conditions which may be considered as extreme in comparison to the physio-chemical characteristics of the normal environment of human cells. These organisms thrive in habitats which are intolerably hostile or even lethal to other forms of terrestrial life. They thrive in extreme hot niches, ice and salt solutions, as well as acid and alkaline conditions, some may grow in toxic waste, organic solvents, heavy metals, or in several other habitats that were previously considered inhospitable for life. They are classified according to the conditions in which they grow. Extremophiles include members of all three domains of life, i.e., bacteria, archaea and eukarya. Most extremophiles are microorganisms, but this group also includes eukaryotes such as protists (e.g., algae, fungi and protozoa) and multicellular organisms. Archaea is the main group to thrive in extreme environments. Although members of this group are generally less versatile then bacteria and eukaryotes.

Psychrophiles

Psychrophiles (psychrotolerant or psychrotroph) are the microorganism that prefers permanently cold environments, but can also tolerate a wide range of temperatures reaching up into the mesophilic range (Cavicchioli and Siddiqui, 2006). Psychrophilic microorganisms have successfully colonized all permanently cold environments from deep sea to mountains. Psychrotolerent microbes are extremely important since they survive and retain their functionality in cold temperature conditions, while growing optimally at warm temperatures. High-altitude cold habitats of the Himalayas are little explored with respect to bacterial diversity. Soil formation and primary microbial succession can be well studied in recently deglaciated areas where phototrophic microorganisms may play a role as primary producers (Frey *et al*., 2013). Psychrophilic microorganisms provide an enormous natural resource of enzymes that function effectively in the cold, and these cold-adapted enzymes have been targeted for their biotechnological potential (Table 1). Cold-adapted enzymes provide economic benefit by being more productive than mesophilic or thermophilic homologues at low temperature, thereby providing energy savings to the processes for which these enzymes are used. Cold-adapted enzymes have found application in industries like household detergents, molecular biology and baking. The biotechnological value of cold-adapted

enzymes stems from their high k_{cat} at low to moderate temperatures, their high thermo-lability at elevated temperatures and their ability to function in organic solvents (Margesin and Feller, 2010).

Table 1: Potential application of extremophiles in biotechnology and industry.

S. No.	Source	Enzymes	Use	Reference
1.	Psychrophiles	Protease	Contact lens cleaning solution, meat tenderizing	Cavicchioli and Siddiqui (2006); Wang *et al.,* (2010)
		Protease, lipase, cellulases, amylases	Detergent	Joseph *etal*. (2008); Collins *etal*. (2005); Wang *et al.,* (2010)
		Alkaline phosphatase	Molecular biology	Dahiya *etal*., (2006)
		Lipases and proteases	Cheese manufacture	Cavicchioli and Siddiqui (2006)
		β - Galactosidase	Lactose hydrolysis in milk products	Cavicchioli and Siddiqui. (2006); Joseph *etal*. (2008)
2.	Thermophiles	Cellulases	Production of alcohol, fruit industry, household chemistry	Antranikian *et al.* (2005)
		Amylases, Pullulanases	Starch processing, glucose and fructose for sweeteners	Alqueres *et al.* (2007); Antranikian *et al.* (2005); Eichler J. (2001)
		Proteases and lipases	Dairy products	Egorova *et al.* (2005); Eichler J. (2001)
		Xylanases	Paper bleaching	Egorova *et al.* (2005); Eichler J. (2001); Andrade *et al.* (2001)
		Protease	Amino acid production from keratins, food processing, baking, brewing, detergents	Egorova *et al.* (2005); Eichler J. (2001)
3.	Halophiles	Carotene	Food colouring	Raj *et al.*, (2007); DasSarma *et al.*, (2010)
		Glycerol	Pharmaceuticals	Lentzen *et al.*, (2006); DasSarma *et al.*, (2010)
		Lipids	Heating oil and cosmetic packing	Bestvater *et al.* (2008)
		Nucleases, amylases and proteases	Flavouring agents	Amoozergar *et al.*, (2008); Fukushima *et al.*(2005); Souza, (2010)
		Membranes	Surfactants for pharmaceuticals	DasSarma *et al.*, (2010)
4.	Alkaliphiles	Proteases, cellulases, xylanases and lipases	Detergents	Ito *et al.* (1998); Horikoshi *et al.* (1973)
		Proteases	Gelatin removal on x-ray film	Fujiwara *et al.* (1991); Ishikawa *et al.* (1993)
		Pectinases	Fine papers, waste treatment and degamming	Yoshihara *et al.* (1982)
5.	Acidophiles	Amylases	Degradation of starch	(Buonocore *et al.* 1976).
		Proteases	Non-allergic preservatives for medicines and cosmetics	Gaffney *et al.* 1996; Honda 1998)
6.	Barophiles	Proteases	High pressure bioreactor systems	Michels *et al.* (1997)

Thermophiles

Thermophiles are the microorganisms which optimally grow between temperatures 60-110^{o}C. The ability of thermophilic bacteria to grow and propagate at elevated temperature and to produce extracellular enzymes with unique and valuable properties was due to their ability to manipulate their genetic composition. Thermophilic

microorganisms may have tremendous potential in future microbial and enzyme technology because their unique ability to function at high temperature enables development of improved or new biotechnology.Thermophiles mostly belong to two phylogenetically very different domains of life which include bacteria and archaea (Stetter *et al.*, 1993 and Stetter, 2006). Generally, it is agreed that in most hydrothermal environments where temperatures range between 50 and 90^{o}C bacteria is dominating in the communities of microorganisms. In environments where temperatures are above 90^{o}C archaea are dominating (Reysenbach and Yernool, 2002). Among the enzymes microbial esterases and lipases are of substantial interest because of their prospective biotechnological application such as the modification of triglycerides for fat and oil industry as shown in Table 1.

Halophiles

Halophiles (from the Greek, hal, meaning sea or salt, and philos, meaning loving) are distinguished by their requirement of high salinity conditions for growth. Halophiles are salt-loving organisms that flourish in saline environments and can be classified as slightly, moderately or extremely halophilic, depending on their requirement for sodium chloride (DasSarma*etal*., 2012). Although most marine organisms are slightly halophilic, but moderate and extreme halophiles which inhabit hypersaline environments with salinity higher than in the sea are generally more specialized microbes. Many halophiles and halotolerant microorganisms can grow over a wide range of salt concentrations, with requirement or tolerance for salts sometimes dependent on environmental and nutritional factors. High osmolarity in hypersaline conditions is deleterious to most cells since water is lost to the external medium. Halophiles generally accumulate high solute concentrations within the cytoplasm to prevent loss of cellular water (Roberts, 2005; Yancey, 2005). These organisms produceacidicproteinsthatcanfunctioninhigh salinity byleft oversolvatedandreducingaggregation, precipitation anddenaturation(Madern *et al.*, 2000). Halophilic microorganisms also produce many stable enzymes including hydrolytic enzymes such as DNAses, lipases, amylases, gelatinases and proteases which are capable of functioning under high saline conditions, which would lead to precipitation or denaturation of most other proteins. Compatible solutes of halophilic bacteria are used in cosmetics and improving hydration properties generally (Bestvater *et al.*, 2008). Industrial uses of compounds present in halophiles such as β-carotene, poly-β-hydroxyalkanoate, exopolysaccharides,etc as shown in Table 1.

Alkaliphiles

Alkaliphiles are the organisms that usually grow between pH 10 and 12 with optimum pH for growth being above 9. Alkaliphilic microorganisms are widely distributed and can be found in almost any environment, even in environments where the overall pH may not be particularly alkaline. The main industrial application of alkaliphilic enzymes (Table 1) is in the detergent industry (Ito *et al.*, 1998). Alkaline protease is used to decompose the gelatinuous coating of x-ray films from where silver is recovered (Fujiwara *et al.*, 1991; Ishikawa *et al.*, 1993). Alkaliphilic microorganisms are divided into two groups: ones that grow only at alkaline pHabove 8 but not at pH 7, and ones that grow not only at alkaline pH but also at neutral ph. For convenience, the former is called an absolute alkaliphile and the latter as facultative alkaliphile.

Acidophiles

Acidophiles are the microorganisms having optimum pH for growth less than 3.Both natural and man-made acidic environments occur in the biosphere, including sulfidic mine areas and marine volcanic vents, the microorganisms that inhabit these habitats are termed ‘acidophiles’. Acidophiles seem to share distinctive structural and functional characteristics including a reversed membrane potential, highly impermeable cell membranes and a predominance of secondary transporters. Once protons enter the cytoplasm, methods are required to alleviate effects of a lowered internal pH. Acidophiles are most widely distributed in the bacterial and archaeal domains (Johnson and Hallberg, 2003). Acidophiles have a highly impermeable cell membrane to restrict proton influx into the cytoplasm to help maintain change in Ph (Konings *et al.*, 2002). Permeability ofprotons through the membrane determines the rate at

which protons leak inward, the balance between proton permeability, proton influx through energetic and transport systems, and the rate of outward proton pumping determines whether a cell can sustain an appropriate PMF. Number of enzymes from acidophiles have application in pharmaceutical and food industry as shown in Table 1.

Basophiles

Barophilic (piezophilic) microorganisms display elevated growth rates at pressures above 1 atmosphere (Zobell and Morita 1957; Yayanos A. A. 1995). From high-pressure, low-temperature deep-sea sediments numerous barophilic microorganisms havebeen isolated and analyzed for their growth characteristics (Kato *et al.*, 1996, 1995). Studies have shown that these extremophiles have been found to modulate gene expression in response to pressure (Bartlett *et al.*, 1989; Kato *et al.*, 1997; Welch *et al.*, 1996). The enzymes like proteases find their industrial application in high pressure bioreactor systems.

Radiophiles

Radiophiles are the microorganisms that are highly resistant to high levels of ionizing and ultraviolet radiation. The genetic engineering and environmental biotechnology aspects of radiophiles have been reviewed by Daly (Daly M.J., 2000). Examplesinclude*Deinococcusradiodurans* (Sandigursky *et al.*, 2004), *Deinococcusradiophilus* (Yun and Lee, 2004), *Thermococcusmarinus* sp. And *Thermococcusradiotolerans* (Jolivet *et al.*, 2004). The remarkable bacterium thatwas first isolated in 1956 is highly resistant to chemicals, oxidative damage, high levels of radiation*Deinococcusradiodurans* (5 Mrad, 3000 times higher than what would kill a human) and dehydration. It contains a spectrum of genes that encode for multiple activities that repair DNA damage. The genes of three putative uracil-DNA glycosylases have been cloned and expressed to determine their biochemical function (Sandigursky *et al.*, 2004).

Conclusion

Extremozymes have great economic potential in many industrial processes (e.g. agriculture, food, feed and drinks, detergents, textile, leather, pulp and paper).The state of Jammu and Kashmir experiences very harsh climatic conditions and presence of number of glaciers and hot springs can provide numerous opportunities to explore the extremozymes from psychrophilic and thermophilic microorganisms which can prove useful for industries. It is strongly believed that discoveries of new extremophiles and genetic engineering of the newly isolated as well as of the currently available extreme microbes will offer novel opportunities for biocatalysis and biotransformations.

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Studies on Growth and Development of *Oxya japonica* (Orthoptera: Acrididae) on *Andropogon sp.* Under Laboratory Conditions

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Abstract
Oxya japonica is one of the important agricultural insect pest which feeds on a few graminaceous species. Laboratory experiments were carried out during 2012-2014 to understand the biology of *O. japonica*. The study on the growth and development of *O. japonica* revealed that the single female laid 3-9 egg pods during the entire life span. The egg pods were yellowish-brown in appearance, each containing on average 25.17±0.52 eggs. The average pre-oviposition period, average oviposition period and average post-oviposition period was recorded to be 6.15±0.49, 17.4±1.55 and 5.15±1.48 days respectively. The average longevity of male and female was 35.99±0.94 and 39.21±0.43 days, respectively. The life history included five instar stages.
Keywords: *Oxya japonica*, oviposition, longevity.

Introduction

Rice is the main staple food for most people of the Asian countries as it is the important source of carbohydrates. The rice grasshopper, *Oxya japonica* is widely distributed throughout – Asia, Africa, Northern Africa and Algeria. It is an oligophagous insect, which feeds on a few graminaceous species. As the name suggests, rice grasshopper usually feeds on rice and is considered as one of the most important agricultural pests causing reduced crop yield (Hollis, 1971).

Life history and life cycle of different species of Orthoptera were studied in the laboratory as well as field conditions by various workers (Sharma and Gupta, 1997). Bhat and Qadri (1999) studied the micro distribution and fidelitic status of Orthoptera populations in grasslands of Dachigam National Park of Kashmir region. Biology and taxonomic parameters of some short horned grasshoppers from sub-shivalik plains of Jammu region were studied by Sharma and Gupta (1997). Further, significant contribution to grasshopper fauna of Kashmir was made by Bei-Beinko and Mishchenko (1951). Mahmood and Yousuf (1999) recorded Oedipodinae (Acrididae: Orthoptera) from Azad Jammu and Kashmir with the description of a new species *J. Orth.*

Various grasshopper species compete with humans for different plant resources all over the world (Dempster, 1963). In Africa, Australia and Asia, the grasshoppers are generally termed as 'locusts' for their aggressiveness, gregariousness and swarm forming behavior. They often cause extensive and serious damage with their potential of invading cropping areas in swarms of millions of individuals leaving behind devasted fields and plantations. Destruction of rice by grasshoppers is a major factor responsible for low level of subsistence in many tropical countries. For managing such pests information on biology is essential and the stadial time of each instar stage should be known which is not available in literature. Also, the control strategies would be ineffective without a comprehensive knowledge of the biology of the insect.

Material and Methods

The present research investigations were conducted under laboratory conditions. The adult grasshoppers and various immature nymphal stages of *O. japonica* were mostly collected from cultivated rice fields and other surrounding vegetation of grasses from different climatic zones of Kashmir province, during months of May-September in the year 2012. The collections of insects was made from 9:00 to 12:00 noon with the help of insect collecting net and were mass reared in cage measuring 112×82×82 cm, which served as the stock culture. Green leaves of *Andropogon sp.* were clipped and placed into 50 ml conical flask filled with water. Two sides of the cage were made of wood, fitted with windows to clear the grasses and transferring the insects. The other two opposite sides were made of glass and wire mesh respectively. The floor of the cage was made of wire mesh provided with six holes each containing the metallic tube, each measuring 11cm in length and 3 cm in diameter, filled with moist sterilized sand which provided pseudo earth for oviposition. The cage was fitted with the temperature apparatus to maintain the constant temperature. Each cage was provided with a number of plant twigs for perching, moulting and for basking. The humidity of the cage was maintained by placing petridish containing moist cotton in the cage.

Eggs taken from cultures were kept in petridish for observing the incubation period. Newly emerged nymphs were transferred to fresh tender shoot kept in glass jars measuring 15×5 cm individually and fed twice per day as per experimentally designed conditions of food at a temperature of 30°C with 75±5 % RH. Total nymphal durations were recorded for each instar based on moulting and mortality.

The adults were sexed by examining the size, as the abdomen of female was slightly larger than the male. A pair of adults was released in each glass jar covered with muslin cloth and secured with rubber band. The host food plant provided in each glass jar was Andropogon. The jars were maintained at 30°C and 65% relative humidity. Adult longevity, pre-oviposition and oviposition period, pre-mating and mating period was recorded for each pair. All observations were replicated ten times upto successive two generations. The collected data were used to compute per cent adult survival, adult emergence, fecundity and fertility.

The statistics parameters i.e mean and standard deviation were obtained using MS Excel software 2007.

Results and Discussion

The mean pre-oviposition period of *O. japonica* varied from 5.8±0.65 days in 2012-13 to 6.5±0.02 in 2013-14. Mean oviposition period was 16.3±0.36 and 18.5±0.35 days respectively, for the two years. Eggs were laid in egg pods, which were barrel in shape with yellowish-brown colour. Egg pods are curved, about 10-12 mm in length and about 5mm in breadth. Mean number of eggs per pod was recorded to be 25.17±0.52. Mean post-oviposition period varied from 6.2±0.01 days in 2012-13 to 4.1±0.03 days in 2013-14 (Table 1).

There were five nymphal instars in the entire life cycle and the total nymphal period was 59.4±1.12 days and 60.3±1.04 days for the two years respectively. The mean head capsule width was found to be 1.08±0.02, 1.90±0.06, 3.06±0.07, 3.15±0.14 and 4±0.01 mm for I, II, III, IV and V instar, respectively. Nymphs were green in colour and active. The mean body length (mm) of different nymphal instars has been recorded to as (6.41±0.02; 6.45±0.04), (7.25±0.25; 7.22±0.36), (13.36±0.36; 13.36±0.36), (21.66±1.06; 20.97±1.03) and (27.57±1.21; 27.23±1.07) during both the years, respectively. On an average the total development period from egg to adult emergence ranged from 75.33±0.95 in 2012-13 to 78.11±1.18 in 2013-14. The female fecundity for the two years was recorded to be 3.3±0.07 and 4.6±0.12 egg pods per female for the two years, respectively (Table 1).

On an average the adult emergence ranged from 70.85±1.15 percent in 2012-13 to 75.21±1.25 percent in 2013-14. Adult longevity of male was 35.32±1.65 and 36.66±0.65 days for the two years respectively, while it ranged for female from 38.90±0.85 days in 2012-13 to 39.52±0.96 days in 2013-14. Adult longevity was more in female than the male. The adult females appeared larger in size than females, with the robust abdomen (Table 1).

Table 1: Life history, growth and development of O*xya japonica* on *Andropogon sp.* (2012-2014)

Parameters	Generation		Range	Mean ± SD
	I	II		
Nymphal period(days)	59.4±1.12	60.3±1.04	58-61	59.85±0.63
Head capsule width (mm ± SD)				
I instar	1.06±0.01	1.1±0.01	1-1.5	1.08±0.02
II instar	1.95±0.15	1.86±0.05	1.5-2.1	1.90±0.06
III instar	3.01±0.01	3.12±0.01	2.5-3.5	3.06±0.07
IV instar	3.25±0.05	3.05±0.10	3-3.9	3.15±0.14
V instar	3.99±0.91	4.01±0.01	3.5-4.2	4±0.01
Total body length (mm ± SD)				
I instar	6.41±0.02	6.45±0.04	6.2-7.0	6.43±0.02
II instar	7.25±0.25	7.22±0.36	7-8.5	7.23±0.02
III instar	13.36±0.36	13.36±0.36	13.1-4.3	13.40±0.06
IV instar	21.66±1.06	20.97±1.03	19.8-2.1	21.31±0.48
V instar	27.57±1.21	27.23±1.07	25.3-7.6	27.4±0.24
Adult emergence (%)	70.85±1.15	75.21±1.25	67-78	73.03±3.08
Total development period (days)	75.33±0.95	78.11±1.18	66-82	76.72±1.96
Adult longevity-female (days)	38.90±0.85	39.52±0.96	36-44	39.21±0.43
Adult longevity-male (days)	35.32±1.65	36.66±0.65	34-42	35.99±0.94
Pre-oviposition period (days)	5.8±0.65	6.5±0.02	5.5-8	6.15±0.49
Ovipositional period (days)	16.3±0.36	18.5±0.35	15-20	17.4±1.55
Post-oviposition period (days)	6.2±0.01	4.1±0.03	3-8	5.15±1.48
Fecundity (eggs pods /female)	3.3±0.07	4.6±0.12	3-9	3.95±0.91
Eggs per pod	25.54	24.8	23-28	25.17±0.52

A progressive increase in size of head capsule and body length was observed in the successive instar nymphs during post embryonic development. The results were in confirmation with those of Chapman *et al.* (1997) who found the similar results in *Z. variegates.* Growth was observed to be rapid during the instar stages, as the insect undergoes moulting. The gradual increase in the body length supports Ademolu and Idowu (2011) observation that there is increase in the microbial load of *Z. variegates* gut as it moults from first instar to adult stage and it enables to accommodate the increase in food consumption during the post embryonic development.

The results suggest that *O. japonica* is a highly fertile species and is fecundity is highly affected by various abiotic factors like food quality, temperature and humidity. The number of instars recorded in the present study was five. The stadial time of lower instars (I – III) was much lower than those of the higher instars (IV – V) and adults, owing to the rapid development of early instars. The total time nymphal period was observed to be 59.85±0.63 days. It is noteworthy that the lower instars (I-III) are easier to control due to the simplicity in their structural organization and physiology. These findings may have major agro-economic importance.

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Common Bacterial Species Associated with Fish

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Abstract
Fish are one of the most beneficial and nutritional resources of human beings. Fish quality and the microbes associated with fish are directly linked to human health. Bacteria invade almost all organs of the fish including skin, gills and gastrointestinal tract etc. Numerous bacteria taxa have been found to be associated with most of the fish. Both marine and fresh water fish are affected by different bacteria species. However, bacterial association also depends on the surrounding water quality as well. Commonly associated bacteria with fish include *Aeromonas spp., Bacillus spp., Pseudomonas spp. Vibrio spp. Flavobacterium,* etc.
Keywords: fish, microbes, bacteria, association

Introduction

With the development of commercial aquaculture, it has become apparent that diseases can be a significant limiting factor. Major bacterial pathogens of fish include the Gram-negative species, *Aeromonas salmonicida*, *Vibrio anguillarum*, *Vibrio salmonicida* and *Yersinia ruckeri*, the etiological agents of furunculosis, vibriosis, cold-water vibriosis and red mouth disease respectively. In addition, *Aeromonas hydrophila* may cause infections in fish and in Aquaculture Research, generally associated with small surface lesions, sloughing of scales, local haemorrhage and septicaemia. All these diseases are common worldwide and produce considerable economic losses during intensive aquaculture of trout and salmon (Austin and Austin, 1999). The results of numerous studies indicate that fish possess bacterial populations on or in their skin, gills, digestive tract, and light-emitting organs. In addition, the internal organs (kidney, liver and spleen) of healthy fish may contain bacteria, but there is debate about whether or not muscle is actually sterile. Before an infection can be established, pathogens must penetrate the primary barrier. The three major routes of infection are through skin, gills (Birkbeck and Ringo, 2005) and Gastrointestinal (GI) tract (Sakai, 1979; Rose *et al.*, 1989)

Diversity of bacteria in fish

Surface microflora

The bacteria from the surface of freshwater fish have been reported to include *Acinetobacter johnsonii* (Gonzalez *et al.*, 2000) *Aeromonads* (notably *Aeromonas hydrophila, A. bestiarum, A. caviae, A. jandaei, A. schubertii,* and *A. veronii biovar sobria* (Gonzalez *et al.*, 2001), *Alcaligenes piechaudii, Enterobacter aerogenes, Escherichia coli, Flavobacterium* (Zmyslowska *et al.*, 2001), *Flexibacter* spp., *Micrococcus luteus*, Moraxella spp., *Pseudomonas fluorescens*, *psychrobacters* (Gonzalez *et al.*, 2000), and *Vibrio fluvialis* (Diler *et al.*, 2000). To some extent, the presence of aeromonads reflected whether or not the water in which the fish occurred was polluted or cleaned (Gonzalez *et al.*, 2001). Bacteria, typical of those in seawater, have been recovered from the surface of marine fish and include *Acinetobacter calcoaceticus, Alcaligenes faecalis, Bacillus cereus, B. firmus, Caulobacter, coryneforms, Cytophaga/Flexibacter, E. coli, Hyphomicrobium vulgare, Lucibacterium (Vibrio) harveyi, Photobacterium angustum, P. logei, Prosthecomicrobium, Pseudomonas fluorescens, P. marina*, and *Vibrio spp.* (Montes *et al.*, 1999). As a result of a detailed numerical taxonomic study of Gram-negative, oxidase-

positive bacteria recovered from sharks, the dominance of vibrios was noted, with representatives including *V. harveyi* and *V. alginolyticus*. Other groups included *Aeromonas, Photobacterium (including P. damselae* and *P. damselae sub sp. piscicida), Alteromonas, Plesiomonas shigelloides, Moraxella,* and *Neisseria* (Grimes *et al.,* 1993).

Gill microflora

Yellow-pigmented, Gram-negative rods, especially *Cytophaga spp.* dominate on gills (Trust, 1975). Aeromonads, coryneforms, enterobacteria, Gram-positive cocci, pseudomonads, and vibrios have also been recovered from the gills of healthy juvenile rainbow trout (Nieto *et al.,* 1984). Gills of fish accommodate *Achromobacter, Alcaligenes, Bacillus, Flavobacterium,* and *Micrococcus* (Shewan, 1961) and yellow-pigmented bacteria, loosely associated with Chryseo bacterium- Flavobacterium - Flexibacter- Cytophaga (Mudarris and Austin, 1988).

Microflora in the digestive tract

Studies on the microflora of the digestive tract have led the way in the use of culture-independent approaches (Huber *et al.,* 2004). However, the bulk of the historical data stems from culturing methods, which will be discussed first. Ringo *et al.* (1995) have written an excellent review on the topic. Initially in the sac fry, only a few taxa (coryneforms and pseudomonas) occur within the digestive tract (Yoshimizu *et al.,* 1980). It is likely that some bacteria become ingested at the yolk-sac stage, leading to the establishment of an initial intestinal microflora (Hansen and Olafsen, 1999). In addition, it has been reported that bacterial colonisation of the digestive tract of turbot larvae coincided with the start of feeding, when the microflora was dominated by Aeromonas and Vibrio (Munro *et al.,* 1994). In an investigation of the intestinal microflora of larval sea bream (*Dicentrarchus labrax*) and sea bass (*Sparus aurata*), it was observed that when the larvae were fed with rotifers, there was a high incidence of *V. anguillarum, V. tubiashii,* and *nonvibrio*. However, feeding with Artemia led to the recovery of mostly *V. alginolyticus, V. proteolyticus, V. harveyi, and V. natriegens*. It was concluded from these experiments that the fluctuations in the dominant components of the microflora reflected the bacteria in the live feed. Indeed, the dominance of vibrios was not recorded until the end of the larval stage (Grisez *et al.,* 1997). The comparative lack of diversity in larvae continues into older fish, and it has been suggested that the flora may be subjected to as-yet undescribed selective effects leading to a restricted number of taxa being present (Liston, 1957).

A comparatively wide range of taxa have been associated with the digestive tract of adult freshwater fish and include *Mycoplasma* (Holben *et al.,* 2002) *Acinetobacter, Enterobacter, Escherichia, Klebsiella, Proteus, Serratia, Aeromonas, Clostridium and Fusobacterium* (Trust and Sparrow,1974). Isolates have been identified by microplate hybridization as *A. caviae, A. hydrophila, A. jandaei, A. sobria,* and *A. veronii* (Sugita, *et al.,* 1994). *Alcaligenes, Eikenella* (Lee and Lee, 1995), *Bacteroides*(Kamei, *et al.,* 1985), *Citrobacter freundii* (Apun *et al.,* 1999), *Hafnia alvei* (Ugajin *et al.,* 1979), Cytophaga/Flexibacter (Nieto *et al.,* 1984), *Bacillus, Listeria, Propionibacterium, Staphylococcus* (Apun *et al.,* 1999), *Moraxella* (Diler and Diler, 1998), and *Pseudomonas*. In one study involving pike perch, it was concluded that *Moraxella* and *Staphylococcus* were unique to the habitat when compared with the digestive tract of other fish species (Diler and Diler, 1989). Modern phenetic and molecular-based studies, including 16S rRNA sequencing have indicated variability in the intestinal microflora of salmonids, notably rainbow trout and Atlantic salmon reflecting the fish farm of origin (Huber *et al.,* 2004), with analyses revealing the dominance of the gamma subclass (Spanggaard *et al.,* 2000) and beta subclass of *Proteobacteria*, and Gram-positive bacteria with a low G + C-content of the DNA (*Carnobacterium*). The approaches have permitted the recognition of potentially new taxa. For example, a 16S rRNA gene sequence with similarity to *Anaerofilum pentosovorans* has been detected. In one detailed study, 41 culturable microbial phylotypes, and 39 sequences from 16S rRNA and 2 from 18S rRNA genes were retrieved from the digestive and

intestinal mucus of rainbow trout and equated largely with *Aeromonadaceae, Enterobacteriaceae (i.e., Buttiauxella, Enterobacter, Hafnia, Pantoea, Plesiomonas,* and *Proteus*) and Pseudomonadaceae representatives. Intestinal contents contained *Arthrobacter, Bacillus, Carnobacterium, Exiguobacterium, Flavobacterium, Kokuria, Microbacterium, Micrococcus, Rhodococcus, Sporocytophaga, and Ultramicrobacterium*. Genomic DNA isolated from intestinal contents and mucus was used to generate 104 random clones, which were mostly affiliated with *Proteobacteria* (>70% of the total). Twelve sequences were retrieved from denaturing gradient gel electrophoresis analysis of the digestive tract or rainbow trout, and dominant bands were mostly related to *Clostridium* (Kim *et al.*, 2007). One of the outcomes of the study was the realization that *Capnocytophaga, Cetobacterium, Erwinia, Porphyromonas, Prevotella, Rahnella, Ralstonia, Serratia,* and *Veillonella* were recognised as occurring for the first time as culturable components of the microflora in the digestive tract of freshwater fish. Using a parallel approach, the digestive tract of wild and farmed salmon from Norway and Scotland were found to be populated with *Acinetobacter junii* and a novel *Mycoplasma* phylotype, the latter of which comprised almost all, i.e., ~96%, of the microflora of the distal intestine of wild salmon Holben *et al.*, 2002). The digestive tract of adult marine fish has been reported to contain *Aeromona, Alcaligenes, Alteromonas, Carnobacterium* (Ringo *et al.*, 2001), *Flavobacterium, Micrococcus, Photobacterium, Pseudomonas, Staphylococcus, and Vibrio*, including *V. iliopiscarius*. Terminal restriction fragment length polymorphism data point to a greater diversity in the posterior compared to the anterior gut in large herbivorous fish, i.e., *Kyphosus sydneyanus* (Moran *et al.*, 2005). Special groups, such as large (gigantobacteria) symbiotic bacteria, have been observed in the digestive tract of surgeonfish from the Red Sea and Indo-Pacific Region (Fishelson, 1999). Also, using a specific nested polymerase chain reaction, methanogens have been detected in the digestive tract and faeces of flounder (*Platichthys flesus*) from the North Sea (van der Maarel, 1999). Indeed, in this study, 16S rDNA sequences revealed 97.6–99.5% similarity to the archaea representative *Methanococcoides methylutens*. Lactic acid bacteria, notably *Carnobacteria*, are common associated with fish, particularly in the digestive tract (Ringo *et al.*, 1998) with investigations highlighting the presence of *Lactococcus* notably *L. lactis* and *L. raffinolactis* (Hagi *et al.*, 2004). To date, studies have emphasised the taxonomy of the organisms, highlighting the presence of *Carnobacterium particularly C. piscicola and C. piscicola* like bacteria (Ringo *et al.*, 2000), and their role as putative probiotics for use in aquaculture. Other lactic-acid bacteria present in the epithelial mucosa have been equated with *Lactobacillus plantarum, Leuconostoc mesenteroides,* and *Streptococcus spp.* In a separate investigation, *Lactobacillus, Enterococcus durans, Lactococcus, Vagococcus, C. divergens,* and *C. piscicola* were recovered from freshwater fish, notably brown trout (Salmo trutta), and characterized phenotypically by numerical analyses (Gonzalez *et al.*, 2000). A previously undescribed species, *C. inhibens*, was recovered from the intestine of Atlantic salmon, and demonstrated antibacterial activity against fish pathogens, notably *Aeromonas salmonicida* and *Vibrio anguillarum* (Joborn *et al.*, 1999).

Diets

Aeromonads, Bacillus, Pseudomonads, and Staphylococcus dominate in diets (Kitao and Aoki, 1976).

Eggs

Healthy eggs are populated by Cytophaga/Flavobacterium and, to a lesser extent, *Pseudomonas* (Bell *et al.*, 1971), reflecting the organisms present in water (Hansen and Olafsen, 1999).

Internal organs

The liver and kidney of healthy turbot have been found to be populated by mostly *Pseudomonas and Vibrio,* including *V. fischeri, V. harveyi, V. pelagius,* and *V. splendidus*. Similarly, *Shewanella spp*. has been recovered

from the internal organs (Decostere *et al.*, 1996). The reasons for the presence of some of these bacteria are unclear. Moreover, it is speculative whether or not the fish are at the earliest stage of an infection cycle.

Biofilms – microbial shelters

In most natural environments, microbes attach to surfaces, multiply and form biofilms which provides enhanced resistance to external disturbances. In this state the biofilm associated cells are 5 more resistant to many toxic substances such as antibiotics, chlorine and detergents (Watnick and Kolter, 2000). The formation of biofilms is usually depicted as a series of discrete stages in life cycle which begins when planktonic cells contact surfaces, either randomly or by chemical attractants. The next steps involve irreversible attachment when cells have multiplied and have started to secrete extracellular polymeric substances such as polysaccharides, proteins and DNA. After that the biofilm maturates and disperses (Simoes *et al.*, 2009). Bacteria living in biofilms are believed to communicate by chemical signalling although orchestrated behaviour of the community is a matter of dispute. A change in gene expression when cells go from planktonic state to biofilms is unquestionable and is the underlying cause of different cell behaviour that characterizes biofilms (Davey and O'Toole, 2000). Although no single mechanism is responsible, many species use quorum sensing to modulate surface attachment, motility, extracellular polymeric production and dispersal (Dunne, 2002). Secreted polymers are defining feature of biofilms but the functions of them are not yet entirely clear. They promote surface attachment and provide structural support but also offer protection from external threats or help secreting strains to grow toward nutrient rich locations (Nadell, 2009, Xavier and Foster 2007). A mature biofilms is usually composed of channels and cavities to allow the exchange of nutrients and waste. Biofilms are not restricted to natural habitats as food processing facilities are ideal environment for biofilm formation where nutrient rich liquid constantly or periodically covers the surfaces. This can cause problem to the production if proper hygienic preventive measures are not performed. Undesirable bacteria such as spoilers (*Pseudomonas spp*.) and pathogens (*Listeria monocytogenes*) have been shown to form biofilms in food processing plants and if the biofilm grows to mature state the threat of persistent contamination of these bacteria in the food is apparent (Bagge-Ravn *et al.*, 2003).

Conclusion

Fish like any other taxa remain associated with numerous bacteria species. The bacteria invade most of the parts of the fish. However association of bacteria with fish also depends on the quality of water in which the fish dwells. Characterization of microbial communities in processing facilities can contribute to better design of hygienic programs and processing equipment that minimizes the risk of accumulation of undesirable microbes in processing surfaces such as spoilage organisms and pathogens.

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Factors Affecting Bioremediation

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Abstract
Bioremediation means using biological agents to clean environment. Increase in the pollution has lead to increase in toxic substances in the environment and being referred to as most effective management tool bioremediation has tremendous future to be called as "Eco biotechnology". Hence we can infer that bioremediation is a attractive tool used at number of sites which were degraded and attained their original position with onset of this technology. Bioremediation technology uses the microbes to remediate contaminated environment and brings back it to original position .Bioremediation has also been a solution for various emerging problems. Several factors affect the process of bioremediation hence these factors play a vital role in the process of Bioremediation.
Key words: Bioremediation, biotechnology, microbes, pollution, remediation factors

Introduction

Bioremediation is concerned with the biological restoration and rehabilitation of contaminated sites and with the cleanup of contaminated areas in more recent times, accidentally or incidentally, as a result of the manufacture, storage, transport, and use of inorganic and organic chemicals (Baker *et al.*, 1994). Bioremediation offers the possibility of degrading, removing, altering, immobilizing, or otherwise detoxifying various chemicals from the environment through the action of bacteria (Sung *et al.*, 2016; Verma *et al.*, 2006 and Boruvka and Vacha, 2006), plants and fungi (Kvesitadze et al., 2006). The advances in bioremediation have been realized through the help of the various areas of microbiology, molecular biology biochemistry, analytical chemistry, chemical and environmental engineering, among others.

Factors effecting bioremediation

The principle of bioremediation is that microorganisms (mostly bacteria or fungi) are used to degrade hazardous contaminants or covert them to less harmful forms. Thus, bioremediation of contaminants is an application of the microbial metabolic activity. Microorganisms, with their enzymatic pathways, act as biocatalysts and facilitate the progress of biochemical reactions that detoxify the targeted contaminants. As a result, bioremediation processes are only applicable in environments that can sustain life. The microbes act upon the contaminants only when they have access to a variety of materials-compounds to help them extract nutrients and energy to build more cells. In very few cases the natural conditions that exist at the contaminated site provide all the essential materials in large enough amount that bioremediation can occur without human intervention - a process called *intrinsic bioremediation*.Frequently, bioremediation needs the construction of engineered systems to supply microbe stimulating materials - a process called *engineered bioremediation*. Engineered bioremediation purely depends on accelerating the desired biodegradation reactions by encouraging the growth of more organisms, as well as by optimizing the environment in which the organisms must carry out the detoxification reactions**.**

The metabolic characteristics of the microorganisms in association with the physicochemical properties of the object contaminants determine whether a specific microorganism - contaminant interaction is possible. The actual successful interaction between the two, however, depends on the environmental conditions of the site of the

interaction. Specific constrains should therefore be fulfilled for a successful bioremediation attempt. These constrains encompass the microbial, chemical and environmental characteristics of the targeted site**.**

Microbial constrains

A lucrativel bioremediation effort relies on the utilization of the appropriate microorganisms (Neilson and Allard, 2008). Such microbial populations can in theory be consortia of naturally existing species or genetically engineered microorganisms. Most applications rely on the use of naturally existing microbial populations which often are not well characterized. That is to say the microbial populations are effective in their desired application but the complete characterization of the population is not well known. This knowledge gap is not necessarily the result of a scientific inability but rather of the continuous dynamic adaptation of the microbial species to their environments. An example of this ability of microbial populations to adapt to the presence of man-made chemicals comes from the field of medicine, where the rapid adaptation of pathogenic organisms and their resulting immunity to specific classes of antibiotics as a result of the excessive use of these antibiotics has been well documented. These adaptational mechanisms advance through selection processes in which variant species with a specific survival advantage for the given environment take over and survive successfully. The survival advantage often relies on the ability of an organism to metabolise as substrate organic molecules (pollutants) existing in a given site. Contemporary microbiological techniques allow the identification of such transconjugants that originate from a background microbial population confirming that such processes are active in bioremediation practice (Berkey *et al.,* 1990). Horizontal transfer of catabolic plasmids among different species existing within a site may also result into species that possess enhanced catabolic or resistance potential.Such plasmid containing bacteria have been separated from polluted sites (Hardman *et al.,* 1986). Species that can through such plasmid transfer catabolise as single carbon source hazardous xenobiotics (as for example 3-chlorobenzoate) have been reported **(**Pertsova *et al.,* 1984**).**

The ultimate impact, however, of such plasmid transfer processes on the field application potential of bioremediation will have to pass through the previously described path of Principles of bioremediation processes natural selection. A newly acquired metabolic advantage will be assessed, through the mechanism of natural selection, and may allow the ultimate successful establishment of a transconjugants species in a contaminated site. Genetically modified microorganisms (GMOs) have often been presented as offering a major potential advantage for bioremediation. The development of recombinant DNA and other genetic engineering technologies, in the late 1970s, was believed that could be widely applied for environmentally-beneficial purposes, including the clean-up of contaminated soil and water (Romantschuk *et al.,* 2000, Singh *et al.,* 2008 and Sayler and Ripp, 2000). The continuously growing knowledge on catabolic pathways and critical enzymes provides the basis for the rational genetic design of new and improved enzymes and pathways for the development effective processes. Many researchers had expected that genetically modified organisms having novel biochemical traits or enzymatic activities would quickly find broad applicability in bioremediation of hazardous chemicals from the environment (Glass, 2005). However the practical impact of GMOs is likely to remain low for many key reasons. Public, economic and technical issues associated with the let go of genetically engineered, or recombinant, microbial species into an open environment usually arise. Many site owners, consultants and regulators are more comfortable choosing technologies and methods with which they are familiar, have a long track record of success and thus a greater predictability. Legislative reasons are associated with the strict control on the release of such organisms into the environment. There is significant concern about the long term survival of genetically engineered species into a natural environment where they would have to compete with the naturally existing consortia that had ample time to adapt to the prevailing environmental conditions. Thus, difficulties in obtaining permission to use genetically engineered microorganisms from government regulatory agencies as well as public controversies have made companies reluctant to develop bioremediation strategies based on GMOs (Glass, 2005 and Wilson, 2005).

Finally, their use is considered costly. Technically speaking, it seems more plausible to use GMOs in *ex-situ* bioremediation treatment schemes in bioreactors, designed for use with defined soil slurries or water streams in tightly controlled environments. Not only does this limit the widespread release of the GMOs in the environment and avoids the problem of competition with indigenous microflora, but also allows the microorganism to be maintained at controlled temperatures and other growth conditions, to be used with relatively well-defined waste streams containing one or a small number of specific contaminants.

The application of the genetically engineered microorganisms in industrial scale bioremediation is not yet prominent. Until today GMOs have not been used in commercial site remediation projects, with few only exceptions (Strong and Wackett, 2005). Most bioaugmentation projects have used naturally-occurring bacteria for which obtaining regulatory approval is relatively easy. However, recently transgenic plants begin to find applicability in commercial phytoremediation projects**.**

Chemical constrains

Bioavailability of contaminants

In order for the pollutants to be amenable to biological degradation they must be bioavailable (Naidu, 2008). Bioavailability is associated to the physical state of the contaminant and the possibility of efficient contact between the microorganism and the contaminant. This contact is best when the microorganism-contaminant interface is maximised. Regarding physical state, microorganisms generally assimilate pollutants from the liquid phase and cannot effectively degrade a pollutant until it desorbs from aquifer solids, diffuses out of nanopores, or dissolves from nonaqueous phase liquids (NAPLs) into the bulk solution. In such cases, the rate of biodegradation can be controlled by the diffusion, desorption, or dissolution rates. Polar, water soluble contaminants are more easily bioavailable. The increase of the contaminant - microorganisms contact surface for hydrophobic contaminants may require the addition of surface active agents. Knowledge of partitioning and rates of transfer of a chemical between its disolved-sorbed-volatile states becomes important in defining its bioavailability. Bioavailability comprises the effects of all the physical and chemical parameters that eventually dictate the potential for the microbial utilisation of a compound and thus its biodegradation potential **(**Alvarez *et al.,* 2005**).**

Biodegradability of contaminants

The success of any bioremediation project depends mainly on the chemical structure of the organic molecules present in the degraded site (Neilson and Allard, 2008**)**. Some structural features of organic compounds that are not common in nature, called ‘‘xenophores’’ (e.g., substitutions of H with Cl, NO2, CN, and SO3 groups), make such molecules difficult to be metabolized by microorganisms. Thus, contaminants that contain such xenophores tend to be recalcitrant to microbial degradation (Alexander, 1999). Table 1, presents the experienced biodegradability potential of different target organic molecules. Numerous mechanisms and pathways have been elucidated for the biodegradation of a wide variety of organic compounds (Neilson and Allard, 2008). All metabolic reactions are mediated by enzymes. These belong to the groups of *oxidoreductases*, *hydrolases*, *lyases*, *transferases*, *isomerases* and *ligases*. Many of the oxygenase enzymes that attack aromatic hydrocarbons have a remarkably wide degradation capacity due to their non specific substrate affinity. For example, toluene dioxygenase is capable of degrading more than 100 different compounds, including TCE, nitrobenzene, and chlorobenzene. Other examples are esterases, which break down ester bonds by the addition of water; depolymerases, which hydrolyze polymers; dehalogenases, which remove halogen atoms such as chlorine and replace them with —OH groups; decarboxylases which remove CO2 groups (i.e., decarboxylation), hydratases which add water to alkenes converting them into secondary alcohols; glutathione S-transferase which transfers the thiol group tochlorinated compounds with concomitant dechlorination; racemases which catalyze L and D-amino acid interconversions and finally CoA-ligase, which adds -S-CoA to fatty acids during beta-oxidation.

Table 1: Biodegradability of various compounds (Suthersan, 1999 and E. P. A., 2006)

Simple hydrocarbons, C1- C15	Very easy
Alcohols, phenols, amines	Very easy
Acids , esters, amides	Very easy
Hydrocarbons, C12- C20	Moderately easy
Ethers, monochlorinated hydrocarbons	Moderately easy
Halogenated and non halogenated volatile organic compounds (Voc`s)	Moderately easy
Halogenated and non halogenated semi volatile organic compounds(Svov`s)	Moderately easy
Hydrocarbons, greater than C20	Moderately difficult
Multichlorinated hydrocarbons	Moderately difficult
PAHS, PCBS, Pesticides and herbicides	Moderately difficult

Properties of other contaminant

Contaminant properties are critical to contaminant-soil interactions, contaminant mobility and to the ability of treatment technologies to remove, destroy or immobilize contaminants. Important contaminant properties include: Solubility in water, dielectric constant, diffusion coefficient, molecular weight, vapor pressure, density and aqueous solution chemistry **(**Sara, 2003**).**

Nutrients: Most in-situ bioremediation methods practiced today rely on the stimulation of indigenous microbial populations at the site of contamination, by addition of appropriate nutrients, principally carbon, oxygen, nitrogen and phosphorus, and by maintaining optimum conditions of pH, moisture and other factors, to trigger increased growth and activity of indigenous biodegradative microorganisms (Fingerman *et al.*, 2005). Nitrogen and phosphorus requirements are often estimated by calculating a carbon to nitrogen to phosphorus ratio C/N/P close to 100/(10 to 5)/1. Many authors report optimum experimental results C/N/P ~70/3/0.6, , 8/1/0.07 (Atlas, 1981), for crude oil bioremediation of different origin. Fertilizers such as paraffinized urea and octylophosphate in C/N/P 100/10/1 respectively have been suggested for optimal growth. Dibble and Bartha (Dibble *et al.* suggest ratio of C/N/P 800/13/1, illustrating that the nutrient requirement is specific to oil-in-water mixtures and needs individual consideration for any case. Suggested C/N values for composting are between 30-40 (Naidu *et al.*, 2008). A detailed excellent review for nitrogen and phosphorous requirements for bioremediation as well as the deterimental effects of excess nutrients can be found in the literature (Walworth *et al.*, 2008). By controlling ground water flow using injection wells or burred perforated pipes (infiltration gallery) nutrients are delivered . In common settings, ground water that is withdrawn from production wells down gradient from the biostimulation zone is amended with the nutrients required for biostimulation, treated if necessary to remove contaminants, and reintroduced to the aquifer up gradient of the biostimulation zone using the injection wells or infiltration galleries. External source of water is required if the flow of withdrawn water is insufficient to control the subsurface flow The rate of nutrient delivery to the biostimulation zone, hence, is often limited by the solubility of the nutrients in water and the reinjection flow rate**.**

Oxygen, air, hydrogen peroxide

In the most of applications, bioremediation is an oxidation process. During oxidation of contaminants, microorganisms extract energy via electron transfer. Electrons are removed from the contaminant and shifted to a terminal electron acceptor which, during aerobic biodegradation, is oxygen. Oxygen concentrations during decomposition of the organic substrate in the subsurface may become reduced (Pichtel, 2007). The availability of oxygen is the major kinetic limitation on aerobic bioremediation due to the low solubility of oxygen in water. This is more intense in the cases of organic molecules with high oxygen demand such as petroleum hydrocarbons. Air,

oxygen, or other oxygen sources (e.g., hydrogen peroxide, ozone) may be added to the infiltration water to promote aerobic biodegradation. Air sparging of water can supply 8 mg/L dissolved oxygen, sparging with pure oxygen can deliver 40 mg/L, while application of hydrogen peroxide can provide more than 100 mg/L oxygen. Therefore, while air sparging is the simplest and most common oxygen delivery technique, the use of oxygen or hydrogen peroxide may speed the bioremediation process and decrease the pumping required. However, in some cases the increased cost and potential explosion hazard associated with pure oxygen supply may limit the applicability of direct oxygen use. On the other hand, application of hydrogen peroxide to *in-situ* bioremediation is limited by its toxicity to microorganisms, its potential for causing aquifer plugging due to the highly reactive nature of hydrogen peroxide resulting in chemical oxidations of organic and inorganic compounds, producing precipitates (Spain *et al., 1989*).

Alternative electron acceptors: In the absence of molecular oxygen, anaerobic microorganisms use other forms of combined oxygen. For example, denitrifying bacteria use nitrate (NO3 -), nitrite (NO2 -), or nitrous oxide (N2O); dissimilatory metal-reducing bacteria use manganese or ferric iron oxides (e.g., MnO2, Fe(OH)3, or FeOO-); sulfate-reducing bacteria use sulfate (SO42-); and methanogens use carbon dioxide (CO2) or bicarbonate (HCO3) as electron acceptors (Fenchel *et al.,* 1995). In cases where oxygen is progressively depleted, electron acceptors are generally used up in a set sequence determined by the appropriate redox potentials of the oxidation reactions under consideration (Remoundaki *et al., 2003)*. Thermodynamic concepts imply the following sequence of electron acceptor utilization:

$$O_2 \rightarrow NO_{3-} \rightarrow Mn^{4+} \rightarrow Fe^{3+} \rightarrow SO^{2-}{}_4 - \rightarrow HCO_{-3}$$

The implication of this thermodynamic analysis is that when the electron acceptor demand is relatively high (e.g., near the source zone), microbial degradation would sequentially deplete the available oxygen, then nitrate, manganese, ferric iron, and sulfate before methanogenesis becomes predominant. Thermodynamic considerations also imply that heterotrophic microorganisms capable of deriving the maximum amount of energy per unit of carbon oxidized would have a competitive advantage over other species, and their respiration mode would become dominant until their specific electron acceptor is used up.

Metal ions: Although some metals are essential in trace quantities for microbial growth, heavily contaminated sites with high concentrations of metal ions in contaminated soil or water usually inhibit the metabolic activity of the cells, thus affecting directly any bioremediation process (Talley, 2005).

Toxic compounds: High aqueous phase concentrations of some contaminants can create toxic effects to microorganisms, even if the same chemicals are readily degraded at lower concentrations. Toxicity prevents or slows down microbial metabolic activity and often prevents the growth of new biomass needed to stimulate rapid contaminant removal. The degree and mechanisms of toxicity vary with specific toxicants, their concentration, and the exposed microorganisms. Some organic compounds are toxic to targeted life forms such as insects and plants and may also be toxic to microbes. These compounds include herbicides, pesticides, rodenticides, fungicides, and insecticides. In addition, some classes of inorganic compounds such as cyanides and azides are toxic to many microbes; however, these compounds may be degraded following a period of microbial adaption (Talley, 2005).

Biogeochemical parameters

Measurements of various biogeochemical parameters such as dissolved oxygen (DO), redox potential, CO2, and other parameters such as NH^{4+}, NO_{3-}, NO_{2-}, $SO_4{}^{2-}$, S^2-and Fe^{2+} will give an indication of the existing (natural or intrinsic) microbial metabolic activity at the site (Suthersan, 1999).

Environmental constrains

Temperature: Microbial metabolism is substantially affected by temperature (Rike, 2008). Most microorganisms grows well in the range of 10 to 38°C. Technically it is extremely difficult to control the temperature of *in-situ* processes, and the temperature of *ex-situ* processes can only be moderately influenced, sometimes with great expense. Although temperatures within the top 10 m of the subsurface may fluctuate seasonally, subsurface temperatures down to 100 m typically remain within 1° to 2°C of the mean annual surface temperature suggesting that bioremediation within the subsurface would occur more quickly in temperate climates (Freeze and Cherry, 1979)

pH: The pH range in which most bioremediation processes works most efficiently is nearly 5.5 to 8. It is no coincidence that this is also the apt pH range for many heterotrophic bacteria, the major microorganisms in most bioremediation technologies. The suitable pH range for a particular situation, however, is site-specific. The pH is influenced by a complex relationship between organisms, contaminant chemistry, and physical and chemical properties of the local environment. Additionally, as biological processes proceed in the contaminated media, the pH may shift and therefore must be monitored regularly. The pH can be adjusted to the suitable range by the addition of acidic or basic substances (i.e., mineral acids or limestone, respectively). However changes in soil pH will influence dissolution or precipitation of soil metals and may increase the mobility of hazardous materials. Therefore, the soil buffering capacity should be evaluated prior to application of amendments (Pichtel, 2007). The effect of pH on permeability of soils and sediments is not fully understood but it seems that soil pH has also significant effect. Soils have a negative permanent charge and a pH-dependent variable charge. Therefore, pH affects soil dispersion and its permeability. A typical volcanic ash soil has a large amount of pH-dependent charge. Its saturated hydraulic conductivity decreases under low and high pH conditions. When the predominant anion is sulphate, hydraulic conductivity does not decrease even at low pH. However, the saturated hydraulic conductivity of soils with montmorillonite and kaolinite at pH 9 is smaller than that at pH 6 (Fukue *et al.,* 2006).

Moisture content-water activity: Moisture is a very important variable relative to bioremediation. Moisture content of soil alters the bioavailability of contaminants, the transfer of gases, the effective toxicity level of contaminants, the movement and growth stage of microorganisms, and species distribution. During bioremediation, if the water content is too high, it will be difficult for atmospheric oxygen to penetrate the soil, and this can be a factor of limiting growth efficiency and determine the types of organisms that can flourish. Various workers in the field have reported that the water content of the soil should be between 20 and 80%. In cases where no extra source of oxygen is being provided (for example, bioremediation of surface contamination), 20% moisture may be adequate; however, if a continuous recirculation system (pipe networks) is being used for deeper contamination, 80% water content would be more appropriate (Talley, 2005). Soil moisture is frequently measured as a gravimetric percentage or reported as field capacity. Evaluating moisture by these methods provides little information on the "water availability" for microbial metabolism. *Water availability* is defined by biologists in terms of a parameter called water activity (*aw*). In simple terms, water activity is the ratio of the system's vapor pressure to that of pure water (at the same temperature) (Suthersan, 1999 and Talley, 2005).

Redox potential: The redox potential of the soil (oxidation-reduction potential, *Eh*) is directly related to the concentration of O2 in the gas and liquid phases. The O2 concentration is a function of the rate of gas exchange with the atmosphere, and the rate of respiration by soil microorganisms and plant roots. Respiration may deplete O2, lowering the redox potential and creating anaerobic (i.e., reducing) conditions. These conditions will restrict aerobic reactions and may encourage anaerobic processes such as denitrification, sulfate reduction, and fermentation. Reduced forms of polyvalent metal cations are more soluble (and thus more mobile) than their oxidized forms. Well-aerated soils have an *Eh* of about 0.8 to 0.4 V; moderately reduced soils are about 0.4 to 0.1

V; reduced soils measure about 0.1 to - 0.1 V; and highly reduced soils are about 0.1 to -0.3 V. Redox potentials are difficult to be measured in the soil or groundwater and are not widely used in the field (Pichtel, 2007)

Mass transfer characteristics: Mass transport characteristics are used to calculate potential rates of movement of liquids or gases through soil and include: Soil texture, unsaturated hydraulic conductivity,dispersivity, moisture content vs. soil moisture tension, bulk density, porosity, hydraulic conductivity and infiltration rate (Sara, 2003; Hillel, 1998 and Hillel, 1998). Site hydro geologic characteristics Hydro geologic factors for consideration include aquifer type, hydraulic conductivity, hydro geologic gradient, permeability, recharge capability, depth to groundwater, moisture content/field capacity, thickness of the saturated zone, homogeneity, depth to contamination, extent of contamination, and plume stability. These are only some parameters that should be factored into the design of any bioremediation system (Suthersan, 1999; Sara, 2003; Hillel, 1998).

Conclusion

Bioremediation is a multidisciplinary technology and successful application requires deep understanding of all the relevant scientific fields and attenuation processes. It seems that now a days we have entered in the most interesting and intense phase of process development. Potentials and limitations of the technology are well documented in many resources from the web, books and research papers. Generic and technical information are given in details. The experience accumulated over the years is promising to design cost effective successful remediation projects.

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Feeding Habits of Decapod Crustaceans

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Abstract
Any aquaculture enterprise to be a success requires an investment of time, effort and resources. Important linkages for efficient finfish and shellfish production are scientific knowledge, organization, managerial efficacy etc. Besides these mammoth scale negotiations there are certain fundamental and immensely important countenance which are essential for the success of any commercial culture. Among others, one of the fundamental aspects is gathering knowledge about the feeding ecology, feeding habits and food preferences of cultivable fin fish and shell fish species. The knowledge of the feeding helps in decreasing the mortality of the cultivable organism. Current knowledge about the feeding ecology of prawns and shrimps is reviewed, with particular reference to their feeding habits based on different methods of gut content analysis with indices such as index of preponderance, index of relative importance, point method, occurrence percentage or frequency of occurrence and volume etc.
Keywords: Feeding Habits, crustaceans, aquaculture, finfish and shellfish production,

Introduction

Considerable published information regarding the feeding ecology of prawns and shrimps is available but still the work on their exact feeding ecology, habit and preference for their choicest food needs to be carried out. There are still many aspects which are not fully understood. It is substantial to have the knowledge about the diet of an animal in its habitat in order to be aware of its nutritional requirements and its interaction with other organisms. Therefore, the studies to evaluate identify and quantify the resources that specie uses (with the help of gut content analysis) provides information on those selected from the choices available from the environment (Williams, 1981). This paper attempts to review the state of knowledge of prawn and shrimp's feeding ecology for the awareness of the budding researchers working in the related field.

Feeding ecology of decapod crustaceans

Chopra (1939) has mentioned that prawns eat all types of food including both living and dead whatever comes in their way. Similarly, Pannikar (1952) has also mentioned that the food of young penaeids consists of algae, minute organisms and organic detritus. According to Gopalakrishnan (1952) *Penaus indicus* feeds mainly on vegetable matter and crustaceans, but its di*et al*so includes molluscs, foraminiferans, polychaetes, hydroids, trematodes and echinoderm larvae. Dall (1968) claimed the Penaeids to be omnivorous scavengers or detritus feeders. Tiews (1976) observed *P. merguiensis* to feed mainly upon phytoplankton and benthic foraminiferans. Lee *et al.* (1980) made field observations on *Macrobrachium* species and confirmed its omnivorous habit through enzyme studies. They further stated that the diet of *Macrobrachium* species include aquatic insects and larvae, algae, nuts, grains, seeds, fruits, small molluscs and crustaceans, fish flesh and fish offal and other animals. They also found the species to be cannibalistic. Chong and Sasekumar (1981) studied the food and the feeding habits of the white prawn (*Penaeus merguiensis*) and found them to be carnivorous-detritivorous, feeding mainly upon large crustaceans and also on organic detritus. Hag (1984) suggested that Penaeid prawn *Penaeus monodon* juveniles feed mainly upon algal material while adults are observed to be opportunistic in their feeding showing preference towards animal protein.

Many workers have observed crustacean decapods to be important predators in tropical coastal environments (Nelson, 1981; Preston *et al.*, 1992 and Heales *et al.*, 1996). Leber (1985) found that areas colonized by aquatic vegetation are important habitats for shrimps, as they get refuge and food by virtue of its fauna associated to the aquatic macrophytes. Growth of red king crab has been extensively studied both by laboratory rearing (Gray, 1963; Weber, 1967; Matsura and Takeshita, 1989). Feeding and growth of red king crab (*Paralithodes camtschaticis*) were studied by Zhou *et al.* (1998) under laboratory conditions. Davis *et al.* (2005) conducted feeding studies on South African mud crab *Scylla serrata* larvae and found that though mud crab larvae were able to thrive well on the *Artemia* nauplii but it was further recorded by them that the overall development, survival and successful metamorphosis was enhanced by the inclusion of rotifers in the food.

The presence of unrecognizable debris during gut analysis of decapods is suggestive of major role of soft parts in their diet (Williams, 1955). The observations made in this context revealed that *Macrobrachium rosenbergii* is an omnivore that feeds mainly on various plant and animal matter (Mary, 1957; Ling, 1969). Bhimachar (1965) and Raman (1967) also recorded *Macrobrachium rosenbergii* to be bottom feeder and an omnivore, its juveniles consuming more diatoms than adults. Further, Bhimachar (1962) found that immature species are mostly found with empty stomachs as compared to mature specimens. Rao (1967) has reported that *Macrobrachium rosenbergii* shows cannibalistic food habits and even eats its own moults and dead eggs. Phytoplankton and benthic macroinvertebrates have been detected in the stomach of decapods including crabs (Muntz *et al.*, 1965); *Macrobrachium carcinus* (Lewis *et al.*, 1966); *Macrobrachium rosenbergii* (Ling, 1969); cray fish (Caine, 1975); *Palaemon* (Inyag, 1978); *Macrobrachium idella* (Jayachandra and Joseph, 1989); Palaemon (Guerao, 1995); *Macrobrachium borelli* (Collins and Paggi, 1998); *Palaemonetes argentinus* (Collins, 1999); *Aristaeomopha foliacea* (Bello and Pipitone, 2002); *Acetes paraguayensis* (Collins and Williner, 2003).

Subramanayam (1963) studied the stomach contents of *Metapenaeus affinis* and found that its diet was predominated by bottom dwellers such as nematodes, foraminiferans and molluscs. The studies on food content of prawns inhabiting the backwaters of Cochin were conducted by George (1972) who reported that small crustaceans form major food items of juveniles, while only a small portion of stomach content consisted of unidentified objects and debris. Abel and Blum (1977) investigated the ecological aspects of freshwater decapod crustaceans. Investigations regarding food and feeding of *Penaeus monodon* have been carried out by several workers (Hag, 1984; Baskar *et al.*, 2013) who reported the animal to be an omnivore preferring crustacean in particular. While studying the feeding habits of *Macrobrachium dobsoni*, *Macrobrachium affinis*, *Macrobrachium monoceros*, *Penaeus monodon* and *Penaeus indicus*, Kuttayama (1974) reported that the food of prawn in general, consist of varying amounts of organic matter mixed with sand and mud. Many workers (Edwards, 1978; Cortes, 1995) have advocated that the diet of prawn includes several elements of benthic community. According to Cohen *et al.* (1976) the zoea of *Macrobrachium* does not ingest phytoplankton and requires animal food for its growth.

Studies on the food and feeding habits of shrimp suggest that their juveniles feed upon zooplankton, *Artemia* nauplii and microalgae (Emmerson, 1984; Yufera *et al.*, 1984) while the adults are epibenthic detritivores, derieving their nutrients from the various forms of detrital ingestion (Darnell, 1967; Rubright *et al.*, 1981). Similarly, both laboratory and field studies were conducted by Chen and Chen (1992) to study the predatory behaviour of juvenile, *Penaeus monodon* upon zooplankton sps. They came up with the view that while *Penaeus monodon* can affectively ingest fairly more zooplankton as compared to their smaller counterparts.

Feeding rhythms and diet of *Farfantepenaeus paulensis* were studied by Soares *et al.* (2005). They observed that the shrimp showed an omnivorous feeding behaviour. Among prey organisms, polychaetes and tanaids were the main groups recorded. Further, consumption of detritus and plant material decreased as shrimp grew. Collins (2005) conducted trophic studies on two freshwater prawns viz. *Macrobrachium borelli* and *Palaemonetes argentinus*. It was found that both prawns consumed similar food items, but differed in their feeding times.

Macrobrachium Borelli stomach fullness was greater during night than day, whereas *Palaemonetes argentinus* foraging activity occurred by day time.

Biology and ecology including the feeding behaviour of shrimp *Aristeus antennatus* has been studied by many workers (Sarda and Demestre, 1987; Cartes, 1994) and it has been observed that *Aristeus antennatus* feeds on highly diverse prey and it is a highly predatory and active species, feeding continuously (Cortes, 1995). Further, the work of Maynon and Cartes (1997) on the daily ration of shrimp *Aristeus antennatus* indicates that it is a benthic but mobile species, preying mainly upon detritvores or small predators. While studying the ingestion rate and feeding behaviour of the peppermint shrimp, *Lysmata wurdemanni* in laboratory, Zhang *et al.* (1998) found that the shrimp consumed significantly more food than at 25^{o}C. It was also observed that the ingestion rate increased with increasing food concentration in all larval stages and development.

Similar studies related to the food and feeding habits of shrimps have been worked upon in *Aristeomorphs foliacea* (Rainer, 1992, Pipitone *et al.*, 1994; Cortes, 1995; Bello and Pipitone, 2002), *Penaeus esculentus* (Wassenberg, 1990), *Acetes paraguensis* (Collins and Williner, 2003), rose shrimp, *Parapenaeus longirostris* (Kapiris, 2003) and in *Acetes intermedius* (Chiou *et al.*, 2005).

The typical carnivorous habit of decapods has been secondarily modified by detrital or planktivore feeding (Nicol, 1932). Prawns inhabiting the benthic region not only feed on living animals and vegetable matter available nearby but also on dead organic matter. Food is grabbed by chelae of thoracic legs and taken into mouth whereas maxillipedes help in further cutting and driving it towards mandibles, where the food is finely mascerated into finer particles fit for swallowing (Villadolid and Villaluz, 1951). Due to diversified diet found for both Penaeidae and Palaemonidae, several studies have been developed aiming at the role of these consumers in the regulation of the meso and macrofauna of aquatic environment (Bell and Caull, 1978; Nelson, 1981, Leber, 1985; Posey and Hynes, 1991).

According to Collins (1999) the palaemonid (*Palaemonetes argentinus*) is omnivorous grazing on phytoplankton and preying upon slow moving benthic macroinvertebrates. Feeding habits of grapsid *Metopograpus thukuhar* were investigated and observed the grapsid to be an opportunistic feeder with a certain degree of behavioural plasticity.

Collins and Wiliner (2003) while examining the stomach content of *Acetes paraguayensis* (Hansen) from two lakes categorized them as omnivorous, feeding mainly on members of littoral-benthic and lotic communities e.g. Algae, rotifers and microcrustaceans (copepods and cladocerans). Oligochaeta and Diptera larvae were found as alternative food sources when available. The food selection as well as the preference of *P. monodon* depends upon the availability of food items in the pond bottom with preference for natural food when available. Shrimps tend to be detritivores when benthic organisms are scarce gaining nutrients from cellulose, lignin, protein, starch, fats, waxes and oils extracted from detritus. The food of *Solenocera choprai* as studied by Dineshbabu and Manisseri (2009) comprised of decapod crustaceans, unidentifiable mass, fish remains, shells of mollusca, polychaete worms, sand, foraminiferans and small crustaceans (other than decapods) in the decreasing order of abundance. In case of adults the annual index of preponderance revealed preference for decapod crustaceans, detritus and fish remains respectively. In case of females the major component of the food comprised of decapod crustaceans. Annual feeding intensity of adult *S. choprai was* found to be highest in February and lowest from June to December. Feeding intensity was found to be highest in immature females followed by spent females. Jimoh *et al.* (2011) found *Macrobrachium vollenhovenii* as omnivorous detritivore feeding on a variety of plankton species e.g. chlorophyta, euglenophyta, xantophyta, chrysophyta, cladocera, copepoda, protozoa, dinoflagellate, diatoms, insect parts and unidentified food items, with chlorophyta and diatoms forming the most important food items. Baskar *et al.* (2013) while studying the food and feeding habits of *Penaeus monodon* found that the Polychaetes were found to be the most predominant food items as evidenced by the presence of setae, jaws, and occasional

body fragments in the preventriculus on the basis of highest Index of Preponderance (IOP) followed by Prawns, Fishes, Detritus, sand, other crustaceans, Molluscs, Forminiferans, and Minor crustaceans. The index of preponderance, index of relative importance, Gastro Somatic index and feeding intensity were found to be higher during monsoon as compared to summer months. The study suggested that *Penaeus monodon* as carnivorous mainly feeding on animal food items irrespective of size. Bakhtiyar *et al.* (2014) categorized *M. dayanum* as detriti-omnivore feeding on both animal and plant matter with detritus as dominant food item. The study was based on the observation of the total 480 specimens of *Macrobrachium dayanum* (*M. dayanum*) categorizing them into four categories based on size and sex. Out of total 480 analyzed specimens 214 (44.58%) guts were found to be empty while about 266 (55.41%) contained food. The frequency of empty stomachs was found to decrease with increase in size. Index of preponderance revealed that detritus was the dominant food item of *M. dayanum* followed by algae. The second most dominant food item i.e. algae was found to decrease with increase in size of animal. After detritus and algae other important food items were found to be insects, sand, annelids, macrophytes, molluscs, unidentified matter, crustacean and rotifers. Lima *et al.* (2014) studied the diet items of *M. carcinus* from Amazon River estuary throughout the year by the analyzing stomach contents. The results indicated that *M. carcinus* be considered as omnivorous species with more animal component as found in other Macrobrachium species. The stomach content analysis was carried out by using the frequency of occurrence (FO), methods of points (MP) and feeding index (FI) revealing that the prawns fed on detritus, animals and plant fragments as the most important food items. *Ratosquilla anomala* was found to be a carnivore by Prasad and Yedukondala (2015) feeding on fishes, crustaceans, cephalopods, plant material, polychaetes, molluscs, and echinoderms in decreasing order. The index of preponderance and index of relative importance was found to be higher during monsoon as compared to other season whereas the food was not found to vary within different size groups with no marked variation in food composition between males and females was recorded.

Similar studies regarding the feeding ecology of Decapoda have been carried out in *Macrobrachium rosenbergii* (Barros and Valenti, 2003a, b; Mohanty, 2003); *Macrobrachium hainanense* (Mantel and David, 2004); *Penaeus duorarum*, *Paraenaeopis atlantica*, *Penaeus kerathurus*, *Penaeus notialis*, *Paraenaeopis longirostris* (Bello-Olusoji *et al.*, 2005); *Macrobrachium lamarrei* (Sharma and Subba, 2005); *Penaeus esculentus* (Hill and Wassenberg, 2006); grooved tiger shrimps (Maslamani *et al.*, 2007); *Macrobrachium amazonicum* (de Araujo and Valenti, 2007); *M. vollenhovenii* (Abayomi *et al.* 2011); *Macrobrachium dayanum* (Bakhtiyar *et al.*, 2012); *Farfantepenaeus subtilis* (Silva *et al.*, 2012); *Crangon hakodatei* (Maher *et al.*, 2013); *Macrobrachium brasiliense* (Melo and Nagasaki, 2013); *Parapontophilus occidentalis* (Hendrickx and Papiol, 2015).

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Biofiltration in Aquaculture Systems

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Abstract
Like all living organisms, fish require a clean environment for optimal growth and survival. As fish respire and metabolize feed, toxic metabolites are released into the water column. Metabolite accumulation increasingly degrades system water quality. If inorganic or organic toxins within the water surpass biologically critical levels, fish growth may become inhibited and mortality increased. To maintain a clean environment in recirculating systems, a combination of mechanical and biological filtration techniques must be employed. Although nitrification can occur throughout the culture system (e.g., in biofilms on pipe and tank walls), the majority of biochemical reactions pertaining to heterotrophic and autotrophic bacteria occur within biofilters. Biofilters are specifically designed for concentrated bacterial attachment and nitrification.
Keywords: Fish, toxic metabolites, biofiltration, heterotrophic and autotrophic bacteria

Introduction

Biological filtration involves the growth and containment of specific microorganisms working as a consortium to maintain a natural and balanced aquatic environment. These organisms consume unwanted contaminants, such as ammonia, nitrite, nitrate and dissolved organics as their food source, breaking them down into water, CO_2 and nitrogen gas. Over the last two decades, aquaculture has gone through major changes, growing from small-scale homestead-level activities to large-scale commercial farming, exceeding landings from capture fisheries in many areas (National Research Council, 1992; Subasinghe *et al.,* 2000; Wing and Malone, 2005). Fish consumption per capita increased 24% from 1970 to 1998, legumes increased 13% as egg and meat consumption had a net decrease (Frazo, 1999). The need to increase aquacultural production is driving the industry toward more intensive practices. In recent years, there has been a growing concern over the impacts of aquaculture operations (Buschmann *et al.,* 1996; Harache, 2002; Naylor et al., 2000; Cranford *et al.,* 2003; Johnson *et al.,* 2004). It is estimated that 85% of phosphorus, 80–88% of carbon, 52–95% of nitrogen (Wu, 1995) and 60% of mass feed input in aquaculture will end up as particulate matter, dissolved chemicals, or gases (Masser *et al.,* 1999). Increasing regulatory pressure focusing on discharges to natural water bodies will force producers to adopt methods that are environmentally friendlier (White et al., 2004). RAS technology can reduce the effluent waste stream by a factor of 500–1000 (Chen *et al.,*1997; Timmons *et al.,* 2001). Thus, recirculating technologies may allow existing operations to upgrade and expand and comply with future regulations. This paper reviews the implications of the changing use of recirculating systems on biofiltration technologies for freshwater and marine systems with the intention of assisting researchers and biotechnologists in selection of research topics.

Materials and Methods

In biological filtration (or biofiltration), there are substrates with a high specific surface area on which nitrifying bacteria can attach and grow. Biofilters break down highly toxic (un-ionized) ammonia- based waste products from fish and fish feed. A biofilter can be constructed of any material (substrate) that has a large surface area that

can support bacterial growth, is non-toxic to filter bacteria and fish, permits free flow of water through the biofilter.

Depending on design and application, biofilters have the ability to accomplish the following functions. . The first three functions are performed by biological means and the last four are done by physical processes that do not depend on living organisms.

- Remove ammonia
- Remove nitrites
- Remove dissolved organic solids
- Add oxygen
- Remove carbon dioxide
- Remove excess nitrogen and other dissolved gasses
- Remove suspended solids

Ammonia and nitrite-nitrogen in the recycled water are oxidized (converted) to nitrite and nitrate by *Nitrosomonas* and *Nitrobacter* bacteria, respectively. *Nitrosomonas* bacteria use ammonia-nitrogen (in both the bacteria use nitrite-nitrogen as an energy source and produce nitrate-nitrogen as a by-product (Tetzlaff and Heidinger, 1990):

$$\text{Ammonia to Nitrite ---- } 2NH_4 + 3O_2 \Longrightarrow 2NO_2 + 2H_2O + 4H$$

$$\text{Nitrite to Nitrate ---- } 2NO_2 + O_2 \Longrightarrow 2NO_3$$

Denitrification is the dissimilative reduction of nitrate (NO_3^-) to nitrogen gas (N_2), through the production of nitrite (NO_2^-) and gaseous nitric oxide (NO) and nitrous oxide (N_2O) intermediates.

$$NO_3^- \Longrightarrow NO_2 \Longrightarrow NO(g) \Longrightarrow N_2O(g) \Longrightarrow N_{2(g)}$$

This process is performed by heterotrophic bacteria under anoxic conditions and uses nitrate as a terminal electron acceptor in the presence of a carbon and energy source. An electron donor is required as a carbon and energy source to fuel the denitrification process.

Since the nitrifying bacteria are aerobic, adequate oxygen must be added (5-8 mg/l is optimal in most systems) to the biofilter. A ratio of 6:1 of oxygen to ammonia produced in the culture tank must be provided in the biofilter for effective removal of the ammonia by the biofilter bacteria. It takes about 4 to 6 weeks for these bacteria to become well established in the biofilter (Tetzlaff and Heidinger, 1990). At this time bacterial populations stabilize at levels that consume and convert most ammonia and nitrite into harmless nitrate.

Denitrifying bacteria

Many different species are capable of denitrification. Especially the genera *Pseudomonas*, *Alcaligenes*, *Paracoccus* and *Bacillus* comprise many denitrifiers (Knowles, 1982). In the natural environment, a complex interaction of physical, chemical and biological conditions governs the predominance of a particular denitrifying species. In view of the large diversity of denitrifiers, denitrification takes place at a wide range of environmental conditions (temperature, salinity, etc.). Unlike nitrification, where the species diversity is narrow, single environmental determinants do not have a measurable effect on denitrification (Greiner and Timmons, 1998; Zhu and Chen, 1999).

Characteristics of the ideal biofilter

Small footprint: The biofilter should occupy as little space as possible. Space allocated for biofilters takes away area that could be used for culture tanks.

Inert materials of construction: All materials used in the biofilters should be non-corrodible, UV resistant, resistant to rot or decay and generally impervious to chemical attack.

Low capital cost: The biofilter must be inexpensive to purchase or build and cheap to transport to the farm location.

Good mechanical strength: The biofilter and its components must be tough enough to withstand the normal wear and tear of a industrial/agricultural environment.

Low energy consumption: The energy cost (usually electricity) to operate the biofilters should be as low as possible.

Low maintenance requirements: The biofilters should be self cleaning with little or no care required for the normal life of the crop.

Portability: The biofilters should be easily movable to facilitate changes in operation of the facility.

Reliability: Ideally the biofilters should have no moving parts that could fail at an inopportune time.

Controllability: It should be easy to change operating variables to assure optimum performance.

Turndown ratio: The biofilters should be able to work under a wide range of water flow rates and nutrient loading levels.

Safety: The biofilters should not have any inherent dangers to either the crop or the owner/operator.

Utility: The biofilters should accomplish all of the goals set forth in beginning of this paper i.e. removal of ammonia, carbon dioxide, BOD, suspended solids etc.

Scalable: A small system should work the same way as a large system. The performance per unit volume should be constant regardless of the size of the system.

Different biofilter systems

Aquatic Plant Systems: Plants are not normally used for the primary biofilter in aquaculture systems. They do however provide a very good sink for the nitrates produced by a well functioning biofiltration system. Some commercially valuable plants grown in hydroponics systems, aquatic plants such as hydrilla, cattails, water hyacinths and duck weed can be used to absorb nitrates and phosphorus from waste water.

Fluidized bed sand filters: Regular sand filters such as the type used for swimming pool filters or potable water filters are virtually worthless as biofilters for aquaculture. The biofilm quickly fills the spaces between the grains and the pressure drop across the filter rises rapidly. Frequent back flushing is required and the active biological film is removed each time. In contrast, fluidized bed filters have been successfully used for aquaculture applications.

Bead filters: They consist of a closed vessel partially filled with small beads of plastic. Usually the vessel is filled with water and the beads float at the top of the vessel. Water flows up through the bed of beads.

RBC (Rotating Biological Contactors): A typical design consists of plates or disks that are attached to a horizontal shaft. The shaft is located at the surface of the water and it is turned at a very slow speed (1-5

rpm). The disks are half submerged in the water at all times. As they rotate, the biofilm attached to the surface of the disk is alternately exposed to air and then submerged in the water (DeLosReyes and Lawson, 1996)

Trickling Filters

Trickling filters typically consist of a packing or media contained in a vessel (Figure 1). The water to be treated is sprayed over the top of the media and collected in a sump underneath the media. The surface of the media or packing provides the substrate for the growth of a biofilm (Kamstra *et al.*, 1998).

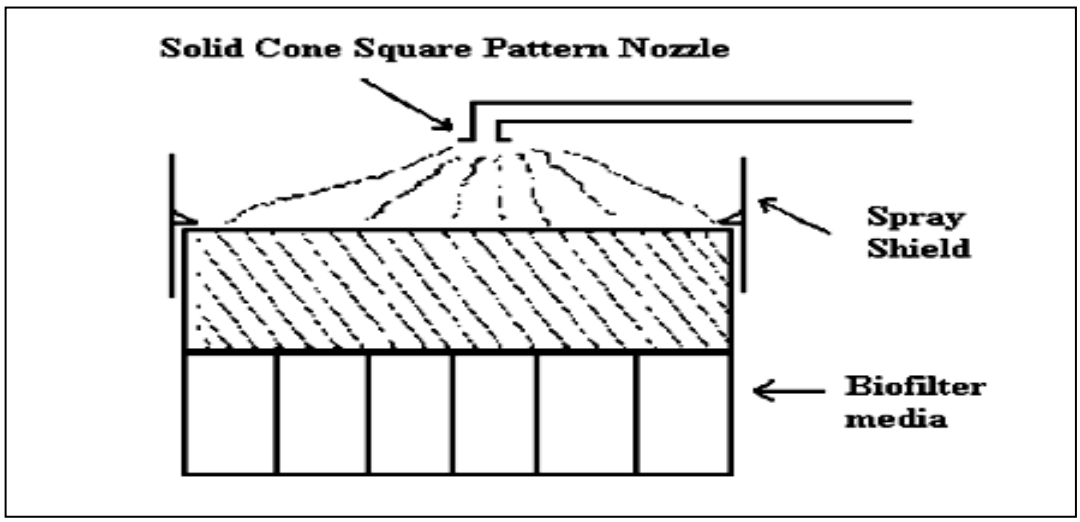

Figure 1: Trickling filter with pressure nozzle distribution system.

Submerged Bed Filters: These filters can be operated in up flow, down flow or cross (horizontal) flow.

Submerged Filters: Submerged filters are excellent choices for small systems because they are very versatile. Ideally the flow path through a submerged filter should be as long as possible. This type of biofilter is known as a long path, plug flow submerged filter. The goal should always be to provide sufficient velocity through the media to insure a fresh supply of oxygen and nutrients to the organisms on the surface of the media (Manthe *et al.*, 1988).

Conclusion

Biofilters are an attached growth process in which a biofilm is generated from the propagation of microorganisms on an inert surface. Biofilters maintain a higher active fraction of biomass, as compared to suspended growth environments, which enables the use of a smaller reactor. The efficient operation and compact size makes biofilters an attractive treatment device for the aquaculture industry, as is illustrated by their wide scale use in the performance of nitrification. Complete nitrogen removal can be achieved in recirculating aquaculture systems through the implementation of a coupled biofiltration treatment scheme employing nitrification and denitrification (Timmons, and JEbeling. Reduction of environmental pollution by using biofilter-recirculating technology is considered an important advantage over other fish culture technologies (Masser, *et al.*, 1999).

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Ecological Functions of Ectomycorrhizal Fungi

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Abstract
Ectomycorrhizae are considered as an integral component of the biology and ecology of soil that has a significant impact on the growth and absorption of nutrients and protection against diseases. ECM fungi are considered as an integral part of plant physiology and play a key role in plant adaptation to specific environmental conditions. This fungus gains carbon and other essential organic substances from the tree and in return helps the trees take up water, mineral salts and metabolites. It also fights off parasites, predators such as nematodes and soil pathogens. Most of the forest trees are highly dependent on their fungal partners especially in areas of poor soil. This association is supposed to be valuable for alleviating nutritional status for both pant and fungi hence is ecologically an important association. The present paper is an attempt to review the ecological functions of ectomycorrhizae.
Keywords: Ectomycorrhizae, nutrient cycling, soil microorganisms, forest ecosystems

Introduction

The roots of terrestrial plants are colonized by mycorrhizae forming symbiotic fungi (Marschner and Timonen, 2005). However taxa belonging to families such as Brassicaceae, Caryophyllaceae, Cyperaceae and Juncaceae are largely non mycorrhizal (Peat and Filter, 1993). Mycorrhizae can be categorized into seven main groups according to their morphology and on the basis of the fungal and the plant taxa forming the symbiosis (Brundrett, 2004). Of these ectomycorrhizae (ECM) are considered an ecological guild distinguished by their stable biotrophic association with the roots of woody plants and the production of macroscopic sporocarps (Luoma *et al.*, 1991). Ectomycorrhizae (ECM) is an association of fungus and feeder roots (root tips) in which the fungus grows predominantly intercellularly in cortical region penetrating the epidermis by secreting proteolytic enzymes and develops extensively outside the root forming a network of hyphae called as mantle which is of variable thickness and color. There is n intercellular infecton forming a network of fungal mycelium around the cortical cells called Hartig net (Sandeep *et al.*, 2015).

Worldwide an estimated 5400 species of fungi form ectomycorrhizal associatios with the most dominant woody plant families like Betulaceae, Fagaceae, Pinaceae, Myrtaceae (Molina *et al.*, 1992). These fungi account for 25% or more of the root mass of forests, thus representing a major below ground structural component of forest ecosystems (Pande *et al.*, 2004). Present paper is an attempt to review the major ecological functions of these Ectomycorrhizal fungi.

Nutrient cycling

Mycorrhizal fungi are major components of nutrient cycling in most of vegetation types. The cycling of nutrients is complex and involves numerous organisms performing processes such as the decomposition of organic and

complex inorganic compounds, the release and assimilation of inorganic minerals and the transport and transfer of these nutrients to host plants. ECM fungi play a primary role within this cycle, supplying nutrients to the host plant outside the depletion zone or mobilizing otherwise inaccessible complex/organic nutrient forms. There is evidence from studies along a north-south transect through Europe (Taylor *et al.*, 2000) that both the proteolytic capabilities and the biodiversity of ECM fungal communities are greater in raw humus soils of northern boreal forests, where nitrification is undetectable, than in more southerly locations where mineral N enrichment occurs, either as a result of natural or anthropogenic inputs.

ECM fungi are known to assimilate nutrients from a range of different sources. Plant litter is a rich source of organic nutrients for which a number of soil organisms, including ECM fungi compete. Within boreal and managed plantation systems, ECM fungi are particularly relevant in the decomposition and release of nutrients from the litter layer. A study by Dames *et al.*, (1998) in South Africa demonstrated the over-accumulation of litter in high altitude *Pinus patula* stands. Furthermore, the results indicate a build-up of immobilized nutrients of N and P and the major cations K, Ca and Mg. It was concluded that litter accumulation was not a result of increased litter production, but rather attributed to low levels of decomposition by organisms such as ECM fungi. Litter/organic decomposition rely heavily on rhizosphere microbial activity and this process is compromised when soil conditions, such as pH, are not optimal for microbial growth.

Decomposition of organic material by saprotrophic microorganisms creates a pool of mineral nutrients that can also be assimilated by ECM fungi. Rock weathering properties of ECM fungi provide direct access to nutrients, providing the ECM plants with a nutritional advantage. ECM host plants have a competitive advantage in terms of nutrient assimilation, as ECM fungi are able to by-pass the traditional nutrient cycle, absorbing nutrients directly from the litter layer without first having to be degraded by saprotrophic organisms (Van Breeman *et al.*, 2000).

Organic matter present in the rhizosphere is derived from plant litter. Both saprotrophic and ECM fungi are able to decompose the organically bound nutrients. Saprotrophic fungi release mineral nutrients into the nutrient pool and mineral nutrients ECM fungi assimilate these nutrients. Nutrient transfer occurs between the two groups of fungi and exudates of soil borne organisms and dead organic matter are the source of organic nutrients. Uncolonized plant roots are able to take up nutrients independently from the pool but are dependent on ECM to gain access to nutrients outside depletion zones.

ECM interaction with soil micro-organisms

Mycorrhizae are thought to potentially have both positive and negative effects on other soil micro-organisms. The ectomycorrhizal mutualism involves interactions with soil microbes as well as with plants. It has been observed that certain bacteria associate with ectomycorrhizae and directly encourage the establishment of mycorrhizal associations. Bacteria known to assist mycorrhizal development are given the general descriptor "mycorrhizal helper bacterium" (Chen *et al.*, 2003; Frey-Klett *et al.*, 1999; Duponnois and Plenchette, 2003). These and other bacteria often associate with the sporocarp (fruiting body) tissue as well as the hyphe (Danell *et al.*, 1993). In a study of the bacterial flora of the ectomycorrhizal fungus *Suillus grevillei* sporocarps, the presence of twenty-seven distinct culturable bacteria species were revealed, the most prevalent genera being *Pseudomonas*, *Bacillus* and *Streptomyces*. Further dual-culture experimentation involving the bacteria isolates and *S. grevillei* showed that the *Pseudomonas* isolates tended to be "helper bacterium." The *Bacillus* strains tend to have little or no effect on the fungus, although some studies have shown them to promote plant growth independently of any interactions with mycorrhizae (Chanway *et al.*, 1996), while *Streptomyces* tended to inhibit the fungus (Luppi-Mosca *et al.*, 1996). Similar observations have been made in a study where bacteria were incorporated in an ectomycorrhizal fungi *Tuber borchii* (Sbrana *et al.*, 2002). Danell *et al.* (1993) suggests that bacterial presence within sporocarps may be incidental and although this was not addressed in the Luppi-Mosca *et al.* (1996) study it would be a logical

explanation for the presence of inhibitory microbes. According to some workers (Hildebrandt *et al*,. 2002; Chen *et al.*, 2003; Parks and Schmitt, 1997) other forms of mycorrhizal symbiosis are affected by various bacteria-mycobiont interactions. Garbaye (1994) suggested that mycorrhizal helper bacteria stimulate ECM fungal germination and enhance ECM formation. Mycorrhizal helper bacteria are also thought to enhance the breakdown of organic nutrients by secreting digestive acids and oxalates, breaking down organic N and C in the soil (Garbaye, 1994). Bacteria have also been identified as possible components of mineral rock weathering associated with ECM (Van Breeman *et al.*, 2000). Saprotrophic and ECM fungi compete for organic nutrient resources in the litter layer. Gadgil and Gadgil (1975) highlighted the importance of the existence of both mycorrhizal and saprotrophic fungi in forest biomes. They demonstrated that there was a decrease in litter decomposition when both trophic groups were present and suggested that this was due to competitive antagonism. Leake *et al.*, (2001), showed antagonistic interaction in microcosms between *Phanerochaete velutina* and *Suillus bovinus* and correlated the interaction with a reduced carbon pulse from the plant to the growing region of the mycelium. This implies a resultant dysfunctional ECM association where normal nutrient exchange is not occurring (Cairney and Meharg, 2002). Lindahl *et al.*, (2001) showed competitive sequestration of phosphorus between a saprotroph, *Hypholoma fasciculare* and an ECM fungus, *Suillus variegatus* and concluded that the potential for either fungus to out-compete depended on the carbon source available to either competitor. ECM fungi are also strong competitors with soil-borne root parasites. The antagonistic modes are both mechanical and biochemical. In the mechanical mode, ECM fungi compete for colonization space in the root and create physical barriers, such as the mantle (Marx, 1973). The biochemical mode involves antifungal compounds released by ECM fungi, as found by Kope *et al.* (1991) in *Pisolithus arrhizus* that suppress pathogenic growth and sporulation. Sen (2001) showed an inhibitory effect of *Suillus bovinus* in association with *Pinus sylvestris* against colonization of pathogenic uninucleate *Rhizoctonia* species. The same inhibitory effects were not observed when the plant host was inoculated with *Paxillus involutus* and *Wilcoxina mikolae*, indicating differences between species in protecting host plants from pathogenic infection. Sen (2001) also found associated *Bacillus* species with *S. bovinus*, suggesting a combined effort of both bacteria and ECM fungi to protect the plant host. The MHB effect has been reported for *Pseudomonas* spp., *Bacillus* spp., *Burkholderia* sp. and *Collimona* species (Garbaye and Duponnois, 1992; Aspray *et al.*, 2006 a, b; Izumi *et al.* 2006; Barbieri *et al.*, 2007; Frey-Klett *et al.*, 2007; Offre *et al.*, 2006). A new mycorrhizal helper bacterium, *Ralstonia* sp., promoting the ectomycorrhizal symbiosis between *Pinus thunbergii* and *Suillus granulatus*, was reported by Kataoka and Futai (2008). *Ralstonia* sp. enhanced hyphal growth of *Suillus granulatus* without cell contact between the mycorrhizal fungus and bacteria, probably because it secreted diffusable substances. This confirms earlier report of Duponnois and Plenchette (2003), who also found no significant correlation between the effect of *Pseudomonas monteilii* strain HR13 on fungal growth under in vitro conditions and mycorrhiza formation presumably due to involvement of different mechanisms in the two processes (Duponnois and Plenchette 2003; Bending *et al.* 2002).

Alternatively, Olsson *et al.*, (1996) found inhibitory effects on bacterial activity in the presence of ECM fungi such as *Paxillus involutus*, *Laccaria bicolor*, *Thelephora terrestris*, *Laccaria proxima*, *Suillus variegatus* and *Hebeloma crustuliniforme*. Bacterial activity was recorded by measuring the incorporation of thymidine. Reduced uptake is probably a result of fungal antibiotic production and competition for limited nutrient resources (Cairney and Meharg, 2002). Contrasting these results, Olsson and Wallander (1998) demonstrated stimulatory effects of *Suillus variegatus* on bacterial activity with amended soil containing biotite nutrient additions. The resultant stimulation/inhibition of bacterial activity as a direct consequence of the nutrient content of the soil supports the argument for potential competition for resources. Olsson and Wallander (1998) also noted effects of ECM fungi on the community structure of the bacteria in close proximity to the host plant roots. To identify the bacteria found associated with ECM fungi, Bowen and Theodorou (1979) isolates from roots of *Pinus radiata* were cultured.

These included three Pseudomonas-type and four Bacillus species. The study found a reduction of ECM colonization of between 42-100% in the presence of these bacteria.

Role of ECM in forest ecosystems

Forest ecosystems are functionally and structurally highly organized systems of biotic and abiotic components, linked into a sensitive dynamic equilibrium (Kraigher, 1999). Being the most common type of mycorrhizae, 125ectomycorrhizal fungi (EMC) have the most important associations with forest trees (Horton *et al.*, 1999; Agarwal and Sah, 2009). They are facultative biotrophs belonging to division of Dikaryomycota, Basidiomycetes and some to Ascomycetes. They are usually found in temperate forests (where soils are used as storage compartments) and sub-temperate forests, but also in boreal, and sub-tropical forests, where Myrtaceae, as well as tropical forest trees can be found (Molina *et al.*, 1992; Wang and Qiu, 2006; He *et al.*, 2009). The abundance of ECM species belonging to Basidiomycetes, namely Hymenomycetes include *Boletus*, *Corinarius*, *Suillus*, *Russula*, *Gomphredries*, *Hebelema*, *Tricholoma*, *Laccaria*, and *Lactarius* and species of Gasteromycetes, e.g. *Rhizopogen*, *Scleroderma*, *Alpara*, and *Pisolithus* all form ectomycorrhizae makes ECM very important in forest ecosystems (Agarwal and Sah, 2009). ECM occurs in the trees like *Pinus*, *Picea*, *Abies*, *Populus*, *Salix*, *Fagas*, *Betula*, *Quercus* and in southern hemisphere trees such as *Eucalyptu*s, *Northofagus*, and *Shorea robusta* of the Dipterocarpaceae family in the monsoon forests of Southeast Asia forming obligatory mycorrhizae (Moore, 2011). Some of them are active in cool or moist areas while others are active in warm or dry locations, and some thrive on coarse woody debris while others prefer humus or other substrate components (Trappe, 1977). These nonspecific characteristics also make ECM globally significant to forest ecosystems. The establishment and performance of outplanted seedlings has often been reported to depend on 125ctomycorrhizal (ECM) fungi (Perry *et al.* 1987; Kropp and Langlois, 1990; Stenström *et al.*, 1990; Pera *et al.* 1999; Baum *et al.*, 2002; Dunabeitia *et al.*, 2004), which may enhance uptake of water and nutrients (Smith and Read 1997) and lengthen the life and increase the growth of roots (Chilvers and Gust, 1982; Wilcox 1996) by protecting them against drought, pathogens, and heavy metal pollution (Chakravarty and Unestam, 1985; Colpaert and Vanassche, 1992; Morin *et al.*, 1999; Van Tichelen *et al.*, 2001; Ortega *et al.*, 2004). The seedlings used in afforestation are often cultivated as bare root seedlings in forest nurseries and reportedly are only colonized by ECM fungi to a lesser extent (Dunabeitia *et al.*, 2004; Menkis *et al.*, 2005). Consequently, failure in afforestation has been hypothesized to be caused by the absence of mycorrhizas (Bjorkman, 1970; Mikola, 1970; Marx, 1980).

The role of mycorrhizas in forest restoration has attracted lot of attention in past (Gaur and Adholeya, 2004; Hidelbrandt *et al.*, 2007). There are at least two mechanisms by which the mycorrhizas in plant roots promote forest restoration. First, as suggested by Galli *et al.* (1994), mycorrhizal colonization in roots plays a role in protecting the plant roots from various stresses, and the second, mycorrhizal colonization of roots increases root surface area for nutrient absorption. In fact, the extraradical mycelia of ECM fungi exploit the greater soil volume and can reach micropore areas and absorb nutrients that may otherwise be inaccessible to plants both physically and chemically (Perez-Moreno and Read, 2000).

Early research on the utility of mycorrhizae in reforestation of mined sites established that prior inoculation of pines with 125ctomycorrhizal fungi improved seedling growth and establishment (Marx, 1977; Marx *et al.*, 1977, Marx *et al.*, 1982). Ectomycorrhizal colonization of root systems is an important factor in determining seedling vigour, and consequently quality (Smith and Read, 1997). Apart from nutritional benefits to their hosts, some mycorrhizal fungi can enable seedlings to withstand high soil temperatures (Marx and Bryan, 1971) and increase resistance to drought (Parke *et al.*, 1983). Of practical importance to nursery management, some mycorrhizal fungi can protect roots against certain pathogens (Sinclair *et al.*, 1982; Sampagni *et al.*, 1985; Stenström *et al.*, 1997) and consequently can improve growth of the seedlings (Smith and Read, 1997). Menkis *et al.*, (2005) investigated

the effect of artificial inoculation on survival and growth of *P. sylvestris* and *P. abies* seedlings. The study showed importance of tree species in picking up the mycorrhizal species as the seedlings of pine and spruce were in most cases colonized by the different fungi. Thus, success of mycorrhizal inoculation in the field largely depends on the fungus, host tree, and ecological conditions of the soil which to a great extent regulate mycorrhizal colonization at a given site (McAfee and Fortin, 1986).

Food for animals

Mycophagy serves to maintain populations of ECM fungi and provides nourishment to small mammals (Malajczuk *et al.*, 1987). Sporocarps are good source of water, protein, carbohydrates and minerals (Johnson, 1994; Claridge *et al.*, 1999). The tripartite relationship between truffles and vertebrates like squirrels and many ground dwelling marsupials and the host tree are well known (Dell, 2002). In this manner ECM also participate in the food chain.

Conclusions

Ectomycorrhizae is a symbiosis between soil fungi and the rootlets of major forest trees and encompasses a wide range of ecological situations. They significantly contribute to a number of ecosystem processes and functions especially nutrient cycling and helps the host trees in survival especially in stress conditions. They also interact with the other soil microorganisms especially the helper bacteria associated with them. The Ectomycorrhizal associations also have proved to be beneficial in forest ecosystems especially in forest regeneration processes.

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Histopathological Impact of Dimethoate Induced Toxicity on Gills for Oxygen Consumption in an Air Breathing Fish *Channa Gachua*

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Abstract
Behavioral alterations like uncoordinated movements, erratic swimming, convulsions, excess mucus secretion, decreased opercular movements, loss of balance, drowning and change in body pigmentation became more apparent with increase in duration of exposure at all test concentration. The results of the water quality of the tap water used in the bioassay are in the normal range and suggest that parameters of the test water were not the cause of fish mortality. However, temperature, hardness, pH, alkalinity and biological factors such as sex, age, health, weight and physiological status are reported to have profound effects on the acute toxicity of pesticides in *Channa gachua*. Toxicity of dimethoate is relatively lower when compared to other air breathing fishes. In the present investigation the histopathological effects of dimethoate in *Channa gachua* were exposed to sublethal concentration of i.e. 1/10th of 96 hour LC_{50} (0.599 ppm) for 30 days for study of histopathology and oxygen consumption. The histopathological studies revealed pathological changes in the gills. The rate of oxygen consumption was also found to be increased initially up to 48 hours then decreased up to end of experiment. The details will be discussed in this paper.
Keywords: Histopathology, dimethoate, oxygen consumption, *Channa gachua*

Introduction

While liberal use of chemical fertilizers and synthetic pesticides helped in ensuring food security to rising population, it inflicted severe injury to the environment especially to the health of soil and aquatic ecosystem. Most pesticides used in agriculture and in hygiene programs are non selective, more or less persistent and bio accumulate in the food chain and pose great danger to the health of non target organism in fresh water. Although mostly pesticides occur at low concentrations in ponds and other water bodies they create serious problems for non target aquatic biota especially the fishes due to their extensive range of biological activity affinity and stability. Fishes are one of the most susceptible animals to pesticide pollution because of their anatomy and physiology. Fishes live in intimate contact with surrounding water through their gills and branchial surface comprises over half the surface area of the body. Only a few microns thick delicate gill epithelium separates the internal environment of fish from external aquatic environment which makes the fish very susceptible to aquatic pollutants respectively. Therefore, contamination of water bodies by pesticides causes acute and chronic poisoning of fish and results in severe damage to vital organs (Singh *et. al.,* 2009). Dimethoate is a broad spectrum systemic organophosphate insecticide active against acaridae, aphididae, aleyrodidae, coccodidea, coleopteran, collembola, diptera, Lepidoptera, pseudococcidae and thyanoptera in cotton, cereals, fruits, vegetables, tea, coffee, tobacco and pastures (Aysal *et. al.,* 2004). Like other organophosphates, dimethoate is an inhibitor of acetyl cholinesterase and causes accumulation of acetylcholine in nerve tissue and effecter organs with the principal site of action being the peripheral nervous system. The accumulation of acetylcholine results in a prolonged stimulation of the cholinergic receptors downstream leading to intense activation of autonomic nervous system, which depending upon the

severity of acetyl cholinesterase inhibition results in tremors, convulsion, respiratory arrest and death. Though the organophosphate pesticide may disappear rapidly from the body either by hydrolysis or elimination, long term and repeated exposure to these pesticides have cumulative effect on fishes respectively. In the aquatic environment the pesticides pollute the ecosystem and find their way into the body of aquatic animals by means of gills, digestive tract and general body surface. Some pesticides accumulate in different tissues of body and produce toxic effects. In fishes it is observed that the organs are affected due to foreign bodies or toxic materials causing loss of equilibrium, irregular movements, and increase in opercular movements, imbalance and finally leading to death. Histopathology deals with the study of pathological changes induced in the microscopical structure of body tissue. Any peculiar alteration of cells may indicate the presence of disease or the effect of toxic substance. In fishes, it is observed that the external organ get affected due to toxic chemical causing irregular movement, loss of equilibrium, increased opercular movement, shedding of scales, lesion on head and gills, finally leading to death.

Materials and Methods

Channa gachua is also known as an air breathing murrel fish belonging to the family channidae of the order channiformes. It is found in estuaries and freshwaters of India. It has a very good flavour and is popular as food. This fish has dual mode gas exchange mechanism as it extracts oxygen from water through gills and from air by accessory respiratory organs (Figure 1). The accessory respiratory organs comprise one pair of supra branchial chambers. The gills of *Channa gachua* had the same basic structure as those seen in most teleost fish four gill arches, each bearing two rows of filaments. Lamellae, the gas exchange units of the gills, projected from both sides of the filaments. The lamellae consisted of the pillar cell system covered by basement membrane and two or three epithelial cell layers. In general, the cells of the inner most epithelial cell layer were flat and those of the outermost cell layer were cuboidal. Mucous and chloride cells were distributed throughout the filament epithelium, which was stratified and contained in 5–7 cell layers as reported earlier by Rahman and Sadhu (2014).

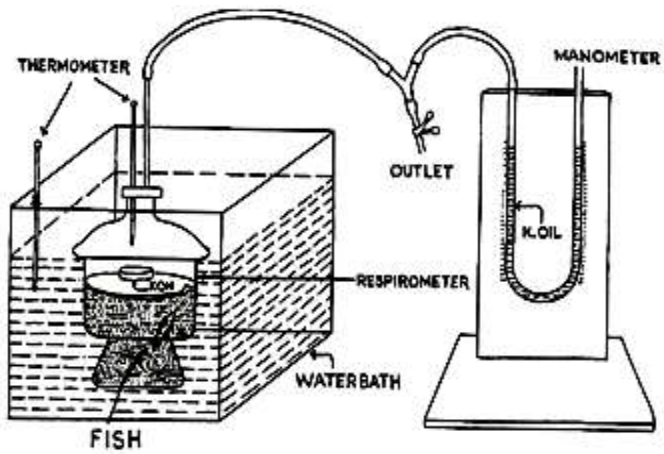

Figure 1: Experimental set up for the measurements of dual mode of oxygen uptake in *Channa gachua.*

Live specimens of *Channa gachua* were procured from local fish dealers at Hazaribag (Latitude 25º 59'N and Longitude 85º 22'E) and maintained in large glass aquaria size (90 x 60 x 60cm) with continuous flow of water. The specimens were fed on chopped goat liver daily during a minimum acclimation period of 15 days in the laboratory. Routine oxygen consumption from air and still water was measured in a closed glass respirometer containing 3 litres of water (initial O_2 content = 6.5 mg O_2/Litre, pH = 7.2) and 0.51 ML of air. The fish had free access to air through a small semi circular hole (10 cm diameter) in a disc float. Carbosorb (B.D.H) or KOH in a petridish placed on the float absorbed CO_2. Thus the fish could exchange gases with water by way of its gills as

well as with the air using the suprabranchial chamber. The air phase of respirometer was connected to a differential manometer. Movement of the manometer fluid follow uptake of oxygen when the CO_2 is absorbed by "Carbosorb" (KOH). The fish were acclimatized to the respirometers for at least 12 hours before the readings were taken. The concentration of dissolved oxygen in the water was estimated by Winkler's volumetric method (Welch, 1948).The oxygen uptake through gills was calculated from the difference between the oxygen levels of the ambient water in the respirometer before and after the experiment and the reading of volume of water in the respirometer. The oxygen uptake from air was measured and calculated from the reading of volume change in the manometer and by the use of the combined gas law equations and vapour pressure. Mean values of oxygen consumption in a series of observations, on each fish at standard temperature pressure dry and standard errors were calculated. The experiments were conducted at 29.0 ± 1.5°C. The pH of the ambient water was measured by an electronic pH meter (Systronics). The respiratory chambers were thermostated by immersion in a temperature controlled water bath. However, sexually mature fishes of almost same weight group (40-50g) were used respectively. For the study of histopathology and oxygen consumption the live test fish were cleaned by using 0.1% $KMnO_4$ to avoid the dermal infection. These fishes are acclimatized in the laboratory for two week prior to the experimentation. Fishes showing normal activity were selected for each test. The test fish, *Channa gachua* were exposed to sub lethal concentration of dimethoate 1/10th of 96 hrs LC_{50} (0.599 ppm) for 60 days. Simultaneously a control set was also maintained. At the end of exposure period the survived fishes were decapitated and immediately the tissues like gills removed and fixed in aqueous Bouin's fluid for 24 hours. These tissues were dehydrated in different grade of alcohol and blocks were prepared in paraffin wax (60-620C). The sections of 5-6 thickness were cut and stained with hematoxyline and Eosin and then mounted in DPX. At the same time rate of oxygen consumption was measured at 7, 15, 30, 45 and 60th days of exposure. Each experiment repeated three times.

Results and Discussion

In the present investigation, the histopathological and oxygen consumption alterations induced by treatment of dimethoate in tissues like gills. The gills of the fish exposed to dimethoate exhibited marked histopathological changes. The main features observed in gills exposed to sublethal concentration of dimethoate were partial degeneration of epithelium of secondary gill lamellae. In some place adjacent secondary gill lamellae appeared to adhere each other. Fusion of secondary gill lamellae resulting in reduction of respiratory surface and vacuolization was also recorded. No change was observed in primary gill lamellae. The effect of dimethoate on gill to different exposure period is shown in plate. The effect of dimethoate on the rate of oxygen consumption in 1, 7, 15, 30, 45 and 60 days exposure period is 0.6242, 0.5815, 0.6566, 0.5812, 0.6347 and 0.6514 in 0.0 ppm but in 0.599 ppm it was 0.5214, 0.7783, 0.8148, 0.6540, 0.5165 and 0.3847 mg/lit/g weight of fish/hour respectively. The similar results were reported by various workers (Ali, 1985; Choudhary and Pandy, 1987), while studied on toxicity of various pesticides on fresh water fish. Srivastava and Srivastva (1980) studied effect of sub lethal concentration of malthion chloride on the histopathology of the gills of *Channa gachua* and observed hyperplasia, hypertrophy vacillation in fish (Figure 2). Kumari and Kumar (1995) and Kumari and Kumar (1997) reported several histopathological changes in kidney, liver, gills, intestine and ovary due to impact of industrial effluents in the fish, *Channa punctatus* and *Hetropneustes fossils*.

Necrosis and destruction of secondary lamellae was noticed after four weeks of exposure. Tilak *et. al.,* (2005) reported that the effect of butachlor technical and machete 50% EC has induced marked pathological changes in fish gills. The changes included the bulging of tips of primary gill filaments, secondary filaments lost their original shape and cutting of secondary gill filaments, pillar cell nucleus showed necrosis and developed vacuoles in the secondary gill epithelium (Figure 3). Malla (1987) studied effect of fenvalerate and cypermethrin on the oxygen consumption of fish, *Cyprinus carpio* and reported that the significant drop in rate of oxygen consumption. Fall in the rate of oxygen consumption in this case was more at higher 3.7 mg/L concentration. Maximum reduction in oxygen consumption was noted at 48 hour and this is because of reduction in physiological activity and damage caused to the gills. Saxena and Chauhan (2003) reported that the decrease in dissolved oxygen caused a stress and resulted in an increase in the rate of oxygen

consumption by the fish while working on oxygen consumption in fish *Labeo rohita* (Ham) caused by distillery effluent. They stated that the inorganic and organic salts might have interfered with respiration in *Labeo rohita* by coagulation of gill mucous and caused asphyxiation as well as inhibition of enzyme system at mitochondrial level. This resulted in decreased in oxygen consumption. Prashant *et. al.,* (2003) studied effect of cypermethrin on toxicity and oxygen consumption in the freshwater fish *Cirrhinus mrigala*, and reported that the decreased in level of oxygen consumption exposed to lethal concentration for 1, 2, 3 and 4 days and also in sublethal concentration of 1, 7, 14 and 21 days. It is may be due to the respiratory dystress as a consequence of the impairment of oxidative metabolism. Aruna and Nagrajan (2007) worked on effects of different ratio of oxygen and water on the survival of gold fish *Carassius auratus* and reported that dissolved oxygen 7.2 mg/ h recorded in all the treatments during the start of the experimental period and latter these parameters gradually decreased at the end of the experimental period. On above literature on the rate of oxygen consumption and histopathology shows that rate of oxygen consumption decreased as concentration of toxicant and time of exposure period increased. It may be due to reduction in respiratory potential of gill tissues probably caused by tissues damage under pesticide tress or it may be due to suppression of metabolic activity of fish at lethal concentrations, dimethoate toxicity like other organophosphate is rapidly reflected in behavioral alterations of exposed fishes. Decrease in opercular rate appears to be an effort of exposed fish to reduce contact of gill epithelium with the poison. To compensate for the loss of oxygen uptake from water fish frequently swims to the surface to gulp air. Increased mucous secretion probably helps in countering irritating effect of dimethoate in skin and mucous membrane. Excitement, hyperactivity and abnormal jerky swimming observed in exposed fishes may be caused by accumulation of neurotransmitter in neuromuscular junction. Loss of balance and drowning reflect the progression towards death as fish succumbs to the continued high exposure of dimethoate. Similar alterations in behavior of dimethoate exposed fish have been reported earlier in *Heteropneustes fossilis* (Pandey *et. al.,* 2009) and *Cyprinus carpio* by Singh *et. al.,* (2009); Rahman and Shamim (2013) in *Channa gachua* reported that gills are vital respiratory organs and cellular damage induced by the metal might impair the respiratory function of the fish by reducing the respiratory surface area. It is concluded that dimethoate is highly toxic to fish which is swiftly reflected in behavioral alterations culminating in death. Further studies on toxicity of dimethoate and its combinations with other pesticides in laboratory and field may help in deciding judicious use of pesticides. Rahman *et. al.,* (2014) in *Channa gachua* reported that the histopathological changes found in gills of the examined fresh water fish are typical for the clinical finding in polluted with heavy metals water of habitat. Influence of water pollution is not only devastating to human being, animals, insects but also aquatic organisms. The more polluted industrial water destroys the aquatic ecosystem and reduced its biodiversity. The decrease in the rate of oxygen consumption after exposure to monocrotophos in *Channa gachua* due to the sluggishness of the fish, as a result of the pesticide stress and also the secretion of excessive mucous, which formed a thin film over the gill there by preventing absorption of oxygen during the process of gaseous exchange. The present study also suggests that, the dimethoate pesticide is very harmful to the aquatic life especially to the fishes, and its urgent need to control this water pollution.

Figure 2: Showing the gills structure with dimethoate in *Channa gachua*.

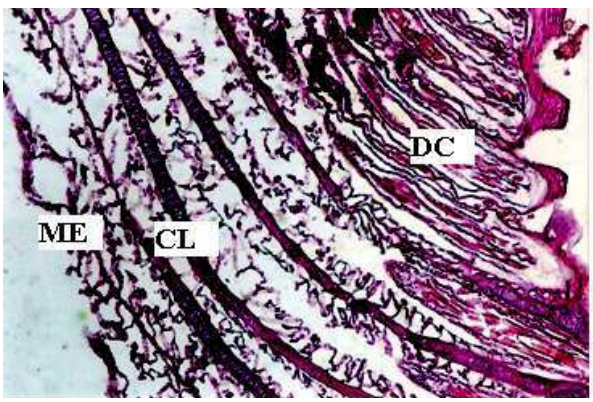

Figure 3: Showing the gills lamella and epithelium in *Channa gachua.*

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