

The Bewildering Taxonomy of Genus *Ranunculus* with Particular Reference to Kashmir Himalaya

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ABSTRACT

Belonging to family Ranunculaceae the genus *Ranunculus* comprises ca. 600 species with a worldwide distribution. The generic delimitation and infrageneric classification of *Ranunculus* have always proved a “Pandora Box” for taxonomists due to its large number of species and high phenotypic plasticity and, therefore, continue to be under discussion. The Kashmir Himalaya, constituting a part of the Northwest Himalaya, represents a repository of the *Ranunculus* species. During the course of present study 25 taxa of *Ranunculus*, belonging to 18 species were recorded from the study area in diverse habitats. The paper highlights the position of the genus at world level in general and at the regional level in particular. In order to remove certain taxonomic confusions some new combinations have been proposed.

Keywords: *Ranunculus*, Kashmir Himalaya, species

INTRODUCTION

The genus *Ranunculus* was first described by Carl Linnaeus in 1753. The Latin name meaning 'little frog' points to the wet habitats in which most of the species grow. It comprises plants commonly known as 'buttercups' for their bowl-shaped flowers with petals having glossy proximal and mat distal portions (Kadota, 1991), although epithets such as 'spearworts', 'water crowfoots' and 'lesser celandine' are also used for other species of the genus. Many of the species are poisonous to cattle and other

livestock when eaten fresh, while some of them are popular ornamentals (e.g. *R. asiaticus*) with cultivars having large and bright-coloured flowers.

Ranunculus L. is a genus of herbaceous annuals and perennials belonging to family Ranunculaceae. It is the largest genus within the family, comprising ca.600 species (Tamura, 1993, 1995; Mabberly, 2008; Srivastava, 2010) and numerous microspecies and apomictic races (Hörandl *et al.*, 2005). Its distribution is almost worldwide and the largest number of species occurs in temperate zones of Europe, Asia, North and South America, Australia, New Zealand, and in the alpine regions of New Guinea (Johansson, 1998). A small number of species occurs in tropical regions where they are restricted to high mountain areas (Tamura, 1993, 1995).

Ranunculus species may be found in a variety of habitats such as forests, dry and damp meadows, marshes, puddles and streams, shallow and marshy banks of rivers and lakes, and alpine heaths. Most of the species appear to have great ecological amplitude; however, habitat-specific species are not uncommon.

Plant architecture is relatively constant within the genus. Many of the species form rosettes or a cluster of basal leaves, from which one or more erect stem axes or runners/stolons emerge. Leaves may be entire, compound or highly dissected. Flowers are single or aggregated into cyme, and are hermaphrodite, usually bright-, dull-, or greenish-yellow. A few species have white (e.g. *R. trichophyllus*, *R. glacialis*) or reddish (*R. asiaticus*) flowers. The calyx consists of (3-)5(-7) sepals and the corolla of (0-)5(-12 or more) petals. The nectary gland, located near the base of the petal, may be naked or covered by a scale. Aestivation of petals may be valvate, imbricate or mixed. Achenes vary from few to many, being smooth, hairy, winged, or with tubercles or hooked spines. The beak may be conspicuous (straight or curved) or inconspicuous.

The remarkable success of *Ranunculus* with respect to species diversity and

distribution is believed to be multifactorial, being mainly attributable to varied permutations and combinations of: (1) high morphological plasticity, including genetic flexibility for rapid adaptation to new habitats, thus permitting development of various eco- and phenotypes; (2) hybridization and polyploidy for diversification, and (3) a broad range of reproductive systems, including vegetative growth, autogamy, allogamy, apomixis and combinations thereof, enabling species to colonize various habitats, especially in regions with colder climates.

Besides hybridization and polyploidy, chromosome repatterning seems to have played a significant role in the formation of new species, as the phenotypic expression of a given gene is often affected by its spatial relations with neighbouring genes in the chromosomes (Goldschmidt, 1940, 1955). The author believes that the formation of new species starts with a large scale mutational event in the chromosomes, a “systemic mutation” which scrambles and rearranges the segments.

The generic delimitation and infrageneric classification of *Ranunculus* have always proved a “Pandora Box” for taxonomists and continue to be under discussion. Previous classifications are based mainly on achene characters (shape of the body and beak, pericarp structure and indumentum), shape of the receptacle, floral morphology (number of sepals and petals, gloss and colour of petals and shape of nectary), life form, and the root system (either uniform or dimorphic with fibrous and tuberous roots). Leaf characters vary considerably within sections (from undivided peltate to strongly dissected), and are often obvious adaptations to habitats, e.g. strongly dissected leaves in water-buttercups (Cook, 1966), and thus of limited value for infrageneric classifications (Hörandl *et al.*, 2005). Infrageneric taxa rarely have exclusive diagnostic morphological characters, but are rather characterized by a combination of features (Hörandl *et al.*, 2005).

In the first worldwide classification system by de Candolle (1818, 1824), based on 159

species, the genus *Ranunculus* L. was classified within tribe *Ranunculeae* and subdivided into five sections, viz. *Batrachium*, *Ranunculastrum*, *Thora*, *Hecatonia* and *Echinella* using features of achenes, roots and flowers. Later worldwide classifications differ considerably among authors. Tamura's surveys (1993, 1995) are the only modern worldwide classifications of family Ranunculaceae based mainly on achene characters. He excluded several small "satellite" genera, viz. *Aphanostemma*, *Arcteranthis*, *Callianthemoides*, *Ceratocephala*, *Cyrtorhyncha*, *Halerpestes*, *Kumlienia*, *Oxygraphis* and *Peltocalathos* (all classified in subtribe *Ranunculinae*; Table 1) previously described under *Ranunculus*. The author subdivided *Ranunculus* s.s. into seven subgenera, viz. *Pallasiantha*, *Coptidium*, *Ficaria*, *Batrachium*, *Crymodes*, *Gampsoceras* and *Ranunculus*; the subgenus *Ranunculus* is divided into 20 sections. Tamura's (1995) classification differs considerably from regional treatments, such as that of Ovczinnikov (1937) for the Flora of USSR, Whittmore (1997) for the Flora of North America, and Tutin and Cook (1993) for the Flora Europaea (Table 1) which reflects the uncertainty about relationships within the genus (Hörandl *et al.*, 2005).

Molecular phylogenies of *Ranunculus* s.l., using Cp DNA restriction site analysis of 78 species (Johansson, 1998), mat K-trn K analysis of 133, mainly European species (Paun *et al.*, 2005), and sequences of the nrITS of ca. 200 species (Hörandl *et al.*, 2005) show a high level of congruence but strongly contradict with previous classifications. The reason for incongruence of molecular data and morphology-based classifications may be the parallel evolution of morphological characters in adaptation to climatic conditions (Hörandl *et al.*, 2005).

Table 1: Position of *Ranunculus* L. and other allied genera in Tamura's survey (1995) and its comparison with some regional treatments (compiled from Hörandl et al., 2005, Lehnebach, 2008).

Tamura (1995) [Worldwide]	Ovczinnikov (1937) [USSR]	Whittemore (1997) [North America]	Tutin and Cook (1993) [Europe]
<i>Tribus Ranunculae</i>			
Subtrib. <i>Trautvetterinae</i>			
<i>Trautvetteria</i>	<i>Trautvetteria</i>	<i>Trautvetteria</i>	
<i>Myosurus</i>	<i>Myosurus</i>	<i>Myosurus</i>	
Subtrib. <i>Ranunculinae</i>			
<i>Kumlienina</i> *		<i>Ranunculus</i> subg. <i>Ranunculus</i> sect. <i>Pseudaphanostemma</i>	
<i>Krapfia</i> *			
<i>Arcteranthis</i> *		<i>R. subg. R. sect. Arcteranthis</i>	
<i>Laccopetalum</i> *			
<i>Halerpestes</i> *	<i>Halerpestes</i> *	<i>R. subg. R. sect. Halodes</i>	<i>R. subg. R. sect. Halodes</i>
<i>Callianthemoides</i> *			
<i>Oxygraphis</i>	<i>Oxygraphis</i> * subg. <i>Euoxygraphis</i>	<i>R. subg. Oxygraphis</i>	
<i>Peltocatathra</i> *			
<i>Cyrtorhyncha</i> *	<i>Ceratocephala</i> *	<i>R. subg. R. sect. Cyrtorhyncha</i>	
<i>Ranunculus</i> *	<i>Ranunculus</i>	<i>R. subg. Ceratocephala</i>	<i>Ceratocephala</i> *
1. subg. <i>Coptidium</i> *	<i>R. subg. Auricomus</i> sect. <i>Coptidium</i>	<i>R. subg. Coptidium</i>	
2. subg. <i>Pallasiantha</i> *	<i>R. subg. Auricomus</i> sect. <i>Coptidium</i>	<i>R. subg. Pallasiantha</i>	<i>R. subg. R. sect. Pallasiantha</i>
3. subg. <i>Ficaria</i> *	<i>Ficaria</i>	<i>R. subg. Ficaria</i>	<i>R. subg. R. sect. Ficaria</i>
4. subg. <i>Batrachium</i> *	<i>Batrachium</i>	<i>R. subg. Batrachium</i>	<i>R. subg. Batrachium</i>
5. subg. <i>Crymodes</i> *	<i>Oxygraphis</i> subg. <i>Crymodes</i>	<i>R. subg. Crymodes</i>	<i>R. subg. R. sect. Crymodes</i>
6. subg. <i>Gampsoceras</i>			
7. subg. <i>Ranunculus</i>			<i>R. subg. Ranunculus</i>
sect. <i>Ranunculus</i>	<i>R. subg. Auricomus</i> sect. <i>Euauricomus</i>	-sect. <i>Epiotes</i>	-sect. <i>Auricomus</i> , sect. <i>Insulares</i>
sect. <i>Flammula</i>	<i>R. subg. Auricomus</i> sect. <i>Flammula</i>	-sect. <i>Flammula</i>	-sect. <i>Flammula</i>
sect. <i>Hecatonina</i> *	<i>R. subg. Hecatonina</i>	-sect. <i>Hecatonina</i>	-sect. <i>Hecatonina</i>
sect. <i>Xanthobatrachium</i>	<i>R. subg. Auricomus</i> sect. <i>Xanthobatrachium</i>	-sect. <i>Hecatonina</i>	-sect. <i>Xanthobatrachium</i>
sect. <i>Thora</i>	<i>R. subg. Thora</i>		-sect. <i>Thora</i>
sect. <i>Aconitifolii</i>			-sect. <i>Aconitifolii</i>
sect. <i>Ranuncella</i>			-sect. <i>Ranuncella</i>
sect. <i>Leucoranunculus</i>			-sect. <i>Leucoranunculus</i>
sect. <i>Physophyllum</i>			-sect. <i>Physophyllum</i>
sect. <i>Acetosellifolii</i>			sect. <i>Acetosellifolii</i>
sect. <i>Chloeranunculus</i>			sect. <i>Ranuncella</i>
sect. <i>Ficatifolius</i>			
sect. <i>Casalea</i> *			
sect. <i>Tuberifer</i>			
sect. <i>Pseudadonis</i>			
sect. <i>Acris</i>	<i>R. subg. Chrysanthi</i>	-sect. <i>Ranunculus</i>	-sect. <i>Ranunculus</i>
sect. <i>Echinella</i> *	<i>R. subg. Pachyloma</i>	-sect. <i>Echinella</i>	-sect. <i>Echinella</i>
sect. <i>Micranthus</i> *	<i>R. subg. Micranthus</i>		-sect. <i>Micranthus</i>
sect. <i>Leptocaulis</i>			
sect. <i>Ranunculastrum</i> *	<i>R. subg. Ranunculastrum</i>		sect. <i>Ranunculastrum</i>

* Genera that have been treated as *Ranunculus* by various authors.

+ Sections or subgenera of *Ranunculus* that have been treated as genera by various

authors Hooker f. and Thomson (1872), in a regional treatment, segregated 21 species of *Ranunculus* into four sections, viz. *Batrachium*, *Ceratocephala*, *Hecatonia* and *Echinella*. In this flora, *R. trichophyllus* is treated as *R. aquatilis* var. *trichophyllus*, and *R. natans* as *R. hyperboreus* var. *natans*. The authors have segregated *R. pulchellus* into three varieties, viz. *typicus*, *sericeus* and *longicaulis*, and *R. hyperboreus* into four varieties, viz. *typicus*, *natans*, *radicans* and *multifidus*. Srivastava (2010) has segregated *R. pulchellus* into three varieties, viz. *pulchellus*, *longicaulis* and *stracheyanum* on the basis of leaf shape, leaf indumentum and stylar beak. In regional Floras, Riedl and Nasir (1993) and Wang and Gilbert (2001) excluded *R.* subg. *Batrachium* and raised it to the level of genus *Batrachium* by following Ovczinnikov (1937), Rostrup (1958) and Löve (1961) and segregated it into two (*B. rionii* and *B. trichophyllum*), and eight (*B. perkenae*, *B. kauffmannii*, *B. rionii*, *B. bungeti*, *B. foeniculaceum*, *B. eradicatum*, *B. divaricatum* and *B. trichophyllum*) species, respectively. It is pertinent to mention that *Batrachium* was given generic status by S. Gray (1821) and A. Gray (1886), while Ascherson and Graebner (1935), Benson (1948) and Clapham (1952) recognized it as a subgenus.

Study area

The Kashmir Himalaya, constituting a part of the Northwest Himalaya, represents a unique biospheric unit (Rodgers and Panwar, 1988). It is situated in the northern fringe of the Indian subcontinent between coordinates of 33° and 37° N latitudes and 72° 30' and 80° 30' E longitudes (Fig. 1). The region consists of a deep elliptical bowl-shaped valley of Kashmir and the cold desert of Ladakh. Zojila (3,529 m) forms the lowest pass on the Greater Himalaya, connecting Kashmir Valley with Ladakh. The Pir Panjal Range bounds the Valley in the south and southwest, while the Korakoram Range guards the Ladakh in the north. The climate of the picturesque Kashmir valley, often called the paradise on earth, is like that of mountains and continental parts of the temperate latitudes.

The temperature ranges from an average daily maximum of 31°C and a minimum of 15°C during summer to an average daily maximum of 4°C and a minimum of -4°C during winter months.



Fig. 1: Map of Jammu and Kashmir

RESULTS AND DISCUSSION

During the course of present study 25 taxa of *Ranunculus*, belonging to 18 species (Table 2; Fig 2), were recorded from the study area in diverse habitats. The specimens were identified using the available literature on floristics such as Hooker's "Flora of British India" (1872), Stewart's "An annotated catalogue of the vascular plants of West Pakistan and Kashmir" (1972), Kachroo, Sapru and Dhar's, "Flora of Ladakh" (1978), Sharma and Kachroo's "Flora of Jammu" vol. 1 (1981), Polunin and Stanton's "Flowers of the Himalaya" (1984), Riedl and Nasir's "Ranunculaceae" In : Flora of Pakistan (1991), Rau's "Ranunculaceae" In : BSI Flora of India (1993); Whittemore's *Ranunculus* In : Flora of North America (1997), Wang and Gilbert's *Ranunculus* In : Flora of China vol. 6 (2001) and Srivastava (2010). The identifications were confirmed by matching the specimens with those deposited in the Kashmir University Herbarium (KASH). The specimens were also sent to Dr. Elvira Hörandl, Department of Systematic and Evolutionary Botany, University of Vienna, Austria, for confirmation of identification.

Table 2: List of the presently studied taxa of *Ranunculus* with population designations and collection numbers of herbarium material.

S. No	Species	Population	Collection No.	Locality
1.	<i>R. arvensis</i> var. <i>arvensis</i>	BGK	031	Batergam, Kupwara: apple orchards on the left side of Kupwara – Chowkibal National Highway, 1618 m.
		URB	025	Boniya, Uri: corn fields along Baramulla - Uri Highway
2.	<i>R. arvensis</i> var. <i>inermis</i> var. <i>nov.</i> *	BGK	036	Batergam, Kupwara: apple orchards on the left side of Kupwara – Chowkibal National Highway, 1618 m.
3.	<i>R. brotherusii</i>	DRK	121	Dras, Kargil: open grassy slopes on the left side of Srinagar Kargil National Highway, 2890 m.
		SKK	170	Sankoo, Kargil: corn fields on the right side of Kargil – Panikhar road, 3290 m.
4.	<i>R. chaerophyllos</i>	TCK	173	Tingochey, Panikhar: open mountainous slope, 3430 m.
5.	<i>R. hirtellus</i> var. <i>hirtellus</i>	GMB	096	Guilmarg : under the shade of <i>Abies</i> and <i>Cedrus</i> on the slopes on the west of Gandola project, 2600 m.
		PKK	112	Panikhar, Kargil: on either side of the Kargil – Panikhar road on moist places under the shade of willow plants, 3250 m.
		RPB	4001	Rastan Pass: open top and slopes on either side of Bandipora-Gurez road, 3650 m.
		TWS	075	Thajwas, Sonamarg : open, bare and moist slopes, 3200m.
6.	<i>R. hirtellus</i> var. <i>gulmargicus</i> var.	GMB	040, 041	Guilmarg : open, moist and damp places of golf course, 2600 m.
7.	<i>R. hirtellus</i> var. <i>emarginatus</i> var. <i>nov.</i>	KDG	045	Kangdori, Guilmarg : open slopes along Kangdori –Apharwat track, 3000 m.
	<i>nov.</i>	TWS	078	Thajwas, Sonamarg : open sandy slopes and moist stream banks, 3200m.
8.	<i>R. hirtellus</i> var. <i>multilobulus</i> var. <i>nov.</i>	GMB	042	Guilmarg: under the shade of <i>Abies</i> along the track leading to high altitude herbal garden of Kashmir University, 2600 m.
9.	<i>R. laetus</i>	ACS	085	Amar Singh College Campus Srinagar: roadsides and unattended land, 1600 m.
		BGK	083	Batergam, Kupwara: along the fence and drains of apple orchards, 1618 m.
		DGB	139	Dawar, Gurez: along Dawar-Tulael and Dawar-Bandipora road, 2800 m.
		OCS	086	Omar Colony, Lawaypora Srinagar: along Srinagar-Baramulla National Highway, 1600 m.
10.	<i>R. lingua</i>	LPS	088	Lawaypora, Srinagar: damps, ditches and puddles on either side of the Srinagar – Baramulla National Highway that remain waterlogged for most part of the year but dry up on the approach of autumn season, 1580 m.
		HSL	090	Hokersar, Srinagar : along the northern and western marshy areas of the lake, 1580 m.
		FSR	177	Hazratbal: on the left side of Hazratbal-Nishat foreshore road about ½ km ahead of Nishat Garden
11.	<i>R. membranaceus</i>	CPL	151	Changla Pas, Leh: moist banks of small streams and channels on the slope facing Leh, 5183 m.
12.	<i>R. munroanus</i>	KDG	081	Kangdori, Guilmarg: under the shade of rocks and in rock crevices along Kangdori – Apharwat track, below gondola ropeway, 3100 m.
13.	<i>R. muricatus</i> var. <i>muricatus</i>	UCS	003	Kashmir University Campus, Hazratbal, Srinagar: on the southern side of GKRS, Hostel, 1580 m.
		BGK	001	Batergam, Kupwara: apple orchards on the left side of Kupwara – Chowkibal National Highway and the nearby paddy fields, 1620 m.
14.	<i>R. muricatus</i> var. <i>emuricatus</i> var. <i>nov.</i>	UCS	050	Kashmir University Campus, Hazratbal, Srinagar: on the southern side of GKRS, Hostel, 1580 m.
		BGK	053	Batergam, Kupwara : apple orchards on the left side of Kupwara – Chowkibal National Highway and the nearby paddy fields, 1618m.
15.	<i>R. natans</i>	PCK	110	Parkachik, Panikhar : water ditch/small pond on the rich side of Panikhar – Padam road, 3220 m.
		PGL	159	Pangong, Leh: a big area of water ditches/puddles giving a marshy appearance, about 10 km ahead of Pangong Lake on the left side of Leh – Pangong road, 4280 m.
16.	<i>R. palmatifidus</i>	GMB	047	Guilmarg: under the shade of <i>Abies</i> along the track leading to high altitude Herbal Garden of Kashmir University, 2600 m.
		ZPS	126	Zojila Pass, Sonamarg: open mountainous slope on the left side of Sonamarg – Kargil Highway, 3600 m.
		TLG	136	Tulael, Gurez: open slopes along Tulael – Masjid Gali road, 2900 m.
17.	<i>R. pulchellus</i> var. <i>pulchellus</i>	PDK	117	Padam, Zaskar, Kargil: marshes, meadows, ditches/puddles on either side of road leading to Padam.
18.	<i>R. pulchellus</i> var. <i>longicaulis</i>	TDK	106	Thrunus, Dras: marshy/water logged land near army camp on either side of Dras – Kargil highway.
		SKK	166	Sankoo, Kargil: along the banks of water channels flowing through corn fields on the right side of Sankoo – Panikhar road, 3290 m.
19.	<i>R. rubrocalyx</i> var. <i>rubrocalyx</i>	KDG	059	Kangdori: on the open slopes along Guilmarg – Kangdori and Kangdori – Apharwat tracks, 2900m.
20.	<i>R. rubrocalyx</i> var. <i>viridiflavus</i> var. <i>nov.</i>	KDG	066	Kangdori: on the open slopes along Guilmarg – Kangdori and Kangdori – Apharwat tracks, 2900m.

21.	<i>R. hyperboreus</i>	SKK	141	Sankoo, Kargil: ditches, puddles and along the banks of shallow and narrow water channels, ahead of Sankoo market on the left side of Kargil – Sankoo road.
		CSL	146	Choglamsar, Leh: ditches, puddles and along the banks of a shallow and narrow water channel on the west of Leh – Manali road, 3200 m.
22.	<i>R. sceleratus</i>	SKS	010	Saidakadal, Srinagar : water ditches on either side of road leading to Hazratbal, 1580 m.
		BGK	029	Batergam, Kupwara: unploughed paddy fields on either side of Batergam – Gushi road, 1618 m.
23.	<i>R. tricuspid var. lancifolius</i>	PGL	153	Pangong: puddles and moist silty banks of ditches about 10 km ahead of Pangong Lake on the left side of Leh – Pangong road, 4280 m.
		CSL	147	Choglamsar: portions of Choglamsar river that remains flooded with water for only a small part of the year but for most part of the year retains little water in puddles and ditches, 3200 m.
24.	<i>R. trichophyllus</i>	KZT	094	Kunzer, Tangmarg : small water stream on the left side of Kunzer – Tangmarg road, 1900 m.
		DGB	133	Dawar, Gurez : small water stream on the right side of Bandipora – Dawar road, about 2 km ahead of Dawar, 2800 m.
		LBS	163	Lasjan Bypass, Srinagar: puddles ditches on the left side of Srinagar – Anantnag National Highway that contain water for most part of the year.
		DMK	020	Drugmulla, Kupwara: unploughed water logged paddy fields on the left side of Kupwara – Baramulla National Highway near army camp, 1618 m.
25.	<i>R. trilobus</i>	BGK	056	Batergam, Kupwara: Lone apple orchard on the left side of Kupwara – Chowkibal National Highway, 1620 m.
		BCS	070	Bemina, Srinagar : along the inner link roads of Bemina Housing Colony near puddles, 1580 m.

* Altitude a.m.s.l; + Author citation for all new combinations in the table is Fayaz, Dar & Wafai

The taxa recorded from the study area were classified into two subgenera (*Batrachium* and *Ranunculus*) and seven sections (*Acris*, *Echinella*, *Flammula*, *Hecatonia*, *Ranunculus*, *Xanthobatrachium* and *Halodes* (Table 3). Of the 18 species, one (*R. trichophyllus*) belongs to subgenus *Batrachium*, while the rest belong to the subgenus *Ranunculus*. Stewart (1972) has reported 36 species from N.W. Himalaya, but during the present investigation some of the species (Table 4) could not be located in the study area. The reasons for not sighting these species could be many. Either the species have shifted/migrated/extirpated due to loss of specific habitats and/or due to overgrazing by cattle, or else they have been misidentified in the said area.

Table 3: Classification of the *Ranunculus* taxa recorded in the present study.

Genus	Subgenus	Section	Species
<i>Ranunculus</i>	<i>Batrachium</i>	<i>Batrachium</i>	<i>R. trichophyllus</i> Chaix
	<i>Ranunculus</i>	<i>Acris</i>	<i>R. laetus</i> Wall ex D. Don
		<i>Echinella</i>	<i>R. arvensis</i> L. var. <i>arvensis</i>
			<i>R. arvensis</i> L. var. <i>inermis</i> var. nov.
			<i>R. muricatus</i> L. var. <i>muricatus</i>
			<i>R. muricatus</i> L. var. <i>emuricatus</i> var. nov.
			<i>R. trilobus</i> Desf.
		<i>Flammula</i>	<i>R. lingua</i> L.
			<i>R. pulchellus</i> C.A. Mey. var. <i>longicaulis</i> (C. A. Mey.) Hook. f. & Thoms.
			<i>R. pulchellus</i> C. A. Mey. var. <i>pulchellus</i>
		<i>Hecatonia</i>	<i>R. sceleratus</i> L.
		<i>Ranunculus</i>	<i>R. brotherusii</i> Freyn.
			<i>R. chaerophyllos</i> L.
			<i>R. hirtellus</i> Royle var. <i>hirtellus</i>
			<i>R. hirtellus</i> Royle var. <i>gulgargicus</i> var. nov.
			<i>R. hirtellus</i> Royle var. <i>emarginatus</i> var. nov.
			<i>R. hirtellus</i> Royle var. <i>multilobulus</i> var. nov.
			<i>R. membranaceus</i> Royle
			<i>R. munroanus</i> Drum. Ex Dunn
			<i>R. palmatifidus</i> H. Riedl
			<i>R. rubrocalyx</i> Regel ex Komarov var. <i>rubrocalyx</i>
			<i>R. rubrocalyx</i> Regel ex Komarov var. <i>viridiflavus</i> var. nov.
		<i>Xanthobatrachium</i>	<i>R. natans</i> C.A. Mey.
			<i>R. hyperboreus</i>
		<i>Halodes</i>	<i>R. tricuspis</i> (Maxim.) var. <i>lancifolius</i> (Bertol.) H. Hara

Table 4: Species of the genus *Ranunculus* L. reported by R. R. Stewart (1972) from N.W. Himalaya that could not be located from the Kashmir Himalaya

S. No.	Name of the species	Stewart's (1972) collection site
1.	<i>R. aucheri</i>	Dras (Ladakh)
2.	<i>R. diffusus</i>	Naoshera (Kashmir), Uri
4.	<i>R. kamchaticus</i>	Dras, Tilel, Sonamarg
5.	<i>R. lobatus</i>	Kargi (Zaskar), Pansi la, Mt. Kolahoi (Kashmir)
6.	<i>R. polypetalus</i>	Rangdum (Zaskar), Masjid Gali (Tilel), Sonamarg, Baltal

Further, a few sites are presently inaccessible to civilians due to security reasons. Stewart (1972) has himself recorded, "*Ranunculus* is another large genus which needs study and revision". Kadota (1991) is also of the opinion that the systematics of the genus is still unsatisfactory. Of the 18 species presently investigated five are of special taxonomic interest as detailed below:

R. arvensis L. hitherto known for having only spiny achenes has, during the present study, been collected with forms having exclusively smooth achenes. The two forms (spiny-achened and smooth-achened) are discrete (Fig 2a), growing sympatrically in BGK population and breed true when seeds are separately sown in pots. In the present study, therefore, the species has been segregated into two varieties, viz. var. *arvensis* (bearing spiny achenes) and var. *inermis* (bearing smooth achenes). In none of the plant both the types of achenes are borne together.

- 1) *R. muricatus* is known for its spiny achenes (Rau, 1993; Whittemore, 1997; Wang and Gilbert, 2001). Blatter and Hallberg (1919) reported for the first time smooth-achened forms within the species and segregated them as a separate species, *R. pseudomuricatus*; while Kak (1981) named such forms as *R. emuricatus*. Riedl and Nasir (1991), Uniyal (2002), Srivastava (2010) have also reported the occurrence of smooth-achened forms in *R. muricatus* from Himalaya and Peninsular India but, without proposing a separate species/subspecies/variatal status, retained them in *R. muricatus*. However, during the course of present investigation it was found that the two forms (spiny and smooth achened) co-exist throughout their distributional range in Kashmir Himalaya (Fig 2f), and in some of the populations the smooth-achened forms even outnumber the spiny-achened ones and the two discrete forms breed true from generation to generation without forming

intermediates in natural populations. Apart from achenes, the two forms differ from each other in foliar indumentum. While in spiny-achened forms the leaves bear continuous/scattered trichomes on both the surfaces, they are glabrous in smooth-achened forms. Neglecting the existence of two discrete forms and clubbing into one species (*R. muricatus*) or segregating the smooth ached forms as a separate species (*R. pseudomuricatus* Blatter and Hallb. or *R. emuricatus* Majeed Kak) seem unjustified; hence during the present study *R. muricatus* was segregated into two varieties viz., var. *muricatus* (spiny-achened) and var. *emuricatus* (smooth-achened).

- 2) *R. hirtellus* is the most variable species amongst Kashmir Himalayan buttercups. The species shows marked variability in both vegetative and reproductive characters. On the basis of these varying characters the species has been segregated into four varieties viz., var. *hirtellus*, var. *emarginatus*, var. *gulmargicus* and var. *multilobulus* (Table 5, Fig 2b-e). Of these, only the type variety (*R. hirtellus* var. *hirtellus*) matches one of the three varieties recognized by Wang & Gilbert 2001, (Table 6); the other three varieties do not match with previously recognized ones and are described as new in this study. Amongst these varieties, var. *gulmargicus* var. *nov.* has the longest flowering period (April-September) *R. rubrocalyx* owes its name to the reddish sepals found in the species. During the course of the present study, however, plants having yellowish-green sepals were found growing sympatrically with those having reddish sepals. These plants have all other characters (vegetative and reproductive) similar to those having reddish sepals. Therefore, the species was segregated into two varieties (var. *rubrocalyx*, having reddish sepals and var. *viridiflavus*, having yellowish-green sepals).

- 3) *R. pulchellus*, which has been segregated into different varieties (*viz. typicus, sericeus, longicaulis and stracheyanum*) by Hook f. & Thomson (1872) and Handel- Mazetti (1939), needs to be studied in detail by making extensive field studies and carrying out molecular characterization, as the species shows tremendous interpopulational variability. The segregation of var. *stracheyanum* on the basis of leaf indumentum seems unjustified as the presence of hairs on the petiole and leaf margins is not a discontinuous character. During the present investigation plants with both the types of leaves (glabrous- and pubescent-petioled) have been observed. Only vars. *longicaulis* and *pulchellus* are distinctly growing varieties of the species in the entire region of Ladakh, the former having narrow elliptic/lanceolate leaves with entire margins while the latter having elliptic leaves with both entire and 2 - 3 lobed margins.
- 4) *R. trichophyllus* grows in two life forms annual and perennial. The former occurs in paddy fields and puddles where water remains available for a few months only during spring season whereas the latter occurs in streams where water flows for the whole year. The two forms differ only in the size of plants. In the former the plants are larger in size with longer internodes while in the latter the plants are smaller with shorter internodes. Some authors (Stewart, 1972 in Flora of West Pakistan; Rau, 1993 in Flora of India; Uniyal, 2002 in Flora of Jammu & Kashmir) have described *R. rionii* from Kashmir Himalaya differing from *R. trichophyllus* mainly in the number of achenes (60-90 in the former and 20-40 in the latter) per achene head. However, during present investigation it was observed that only *R. trichophyllus* grows in Kashmir Himalaya (Fig 2g). Besides, segregating a species, which otherwise could demand at the most a

varietal status, merely on the basis of one character (no. of achenes per head) seems unjustified.

R. trilobus hitherto reported in India from Uttarakhand and Sikkim only, is reported first time during the present investigation growing at a few places in Kashmir valley (Fig 2h).

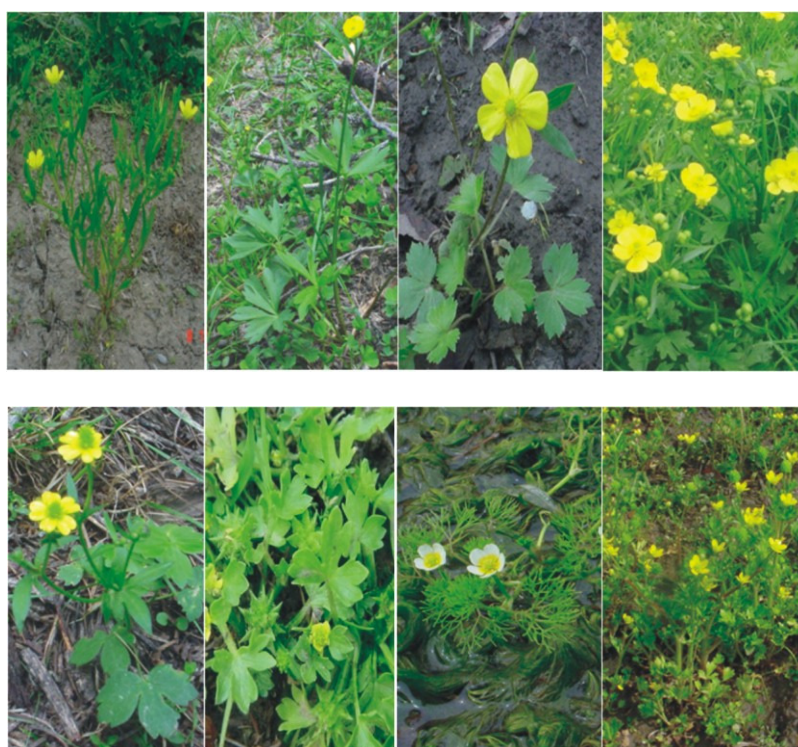


Figure 2. a: *Ranunculus arvensis* with magnified views of spiny and smooth achene heads superimposed; b: *R. hirtellus* var. *hirtellus* with magnified view of achene superimposed; c: *R. hirtellus* var. *multilobulus* var. *nov.* with magnified views of achene and leaf superimposed; d: *R. hirtellus* var. *gulgargicus* var. *nov.* with magnified view of achene superimposed; e: *R. hirtellus* var. *emarginatus* var. *nov.* with magnified view of flower superimposed; f: *R. muricatus* with magnified views of spiny and smooth achene heads superimposed; g: *R. trichophyllus* with magnified view of achene head superimposed; h: *R. trilobus* with magnified view of achene superimposed.

Table 5: Morphological and other features of the varieties of *R. hirtellus* from Kashmir Himalaya.

Attribute	<i>R. hirtellus</i> var. <i>hirtellus</i>	<i>R. hirtellus</i> var. <i>emarginatus</i> var. <i>nov.</i>	<i>R. hirtellus</i> var. <i>gulgargicus</i> var. <i>nov.</i>	<i>R. hirtellus</i> var. <i>multilobulus</i> var. <i>nov.</i>
Stem	15 - 30 cm, branched, pubescent	10 - 15 cm, branched or simple, pubescent	15 - 25 cm, branched, glabrous,	15 - 30 cm, branched, pubescent
Radical leaves	Blade 2.4-6.0 x 3.0-9 cm, 3-sect, middle segment 3-5 lobed, pubescent	Blade 1.3-2.6 x 1.7-3.7 cm, 3-sect, middle segment 3-lobed or rarely without lobes, pubescent	Blade 2.6-3.6 x 4.5 cm, 3-sect, middle segment 3-5 lobed, glabrous	Blade 3.0 x 6.5-3.8 x 8.5 cm, 3-partite to 3-sect, middle segment with more than 10 lobes, pubescent
Floral diameter	1.4 - 2.0 cm	0.9 - 1.4 cm	1.2 - 1.8 cm	1.6 - 2.2 cm
Petal apex	Rounded or flat	Cleft *	Rounded or flat	Rounded or flat
Torus	Pubescent	Pubescent	Pubescent	Pubescent
Carpels/achenes	Pubescent	Pubescent	Glabrous	Pubescent
Flowering/fruiting	May-July	May-July	April-September	May-July
Collection site	<u>Razdan Pass</u> <u>Gulmarg, Panikhar,</u> <u>Kangdori</u>	<u>Kangdori</u>	Gulmarg	Gulmarg

Table 6: Morphological features of the varieties of *R. hirtellus* (after Wang and Gilbert, 2001).

Attribute	<i>R. hirtellus</i> var. <i>hirtellus</i>	<i>R. hirtellus</i> var. <i>humilis</i>	<i>R. hirtellus</i> var. <i>orientalis</i>
Stem	15-27 cm, pubescent, branched or simple	7-14 cm, pubescent, branched or simple	4.5-15 cm, pubescent, branched or simple
Radical leaves	Petiolate, petiole 1-9.5 cm, blade 1.3-3.5 x 1.6-4.2 cm, 3-sect.	Petiolate, petiole 1-5 cm, blade 3-sect or 3-partite, sometimes 3-fid, 0.5-1.5 x 0.7-2.0 cm	Petiolate, petiole 1-5 cm, blade 3-sect or 3-partite, sometimes 3-fid, 0.5-1.4 x 0.8-2.0 cm
Floral diameter	1.2 - 1.5 cm	0.9 - 1.1 cm	0.9-1.2 cm
Torus (receptacle)	Pubescent	Pubescent	Glabrous, rarely with a few hairs
Achene	Pubescent	Pubescent or glabrous	Glabrous
Flowering/fruiting	May-June	July-September	June-August
Distribution	China, Kashmir (3000 - 4000 m)	China (alpine meadows rocks) (4000 - 4800 m)	China (alpine meadows) (3000 - 5000 m)

From the aforesaid account, it is amply clear that the genus *Ranunculus* L. lacks a commonly accepted infrageneric and/or infraspecific classification, both at the world and at regional levels. In the present work an attempt has been made to remove the confusions at regional level by proposing certain new combinations.

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Understanding the Cause of Fish Kill in Nigeen Lake

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ABSTRACT

Occurrences of fish kills are increasing in aquatic ecosystems worldwide, and have been attributed to natural phenomena, as well as human modification and pollution of terrestrial and aquatic environments. Thousands of fish were reported dead mysteriously in Nigeen Lake in August 2012, creating panic in the people of Kashmir Valley. The present investigation was carried to assess which fish species were killed and to determine the ecological conditions in the lake that lead to the fish kill. A combination of factors such as high temperature, low dissolved oxygen, summer kill phenomena and Cyanobacterial bloom lead to mass mortality of fish. Nigeen Lake was also compounded with upsurge of inorganic and organic matter. Ammonia, Total Phosphorus, Chloride and pH showed some rise at the time of fish kill. At some places, the dissolved oxygen level fell to meager 0.4-1.6 milligrams per litre which proved fatal for some fish species like *Crossocheilus* *Diplochilus* which died in large number. After comprehensive scientific tests, we found that many different mechanisms worked synergistically to cause fish kill.

Keywords: Fishkill, temperature, dissolved oxygen

INTRODUCTION

Fish kills occur in virtually every aquatic environment worldwide from a wide variety of natural and human-induced causes. Natural causes include extreme temperature fluctuations, starvation, and disease and low dissolved oxygen (Venugopalan *et al.* 1998). Human-induced kills include known and accidental additions of sewage/organics, pesticides, acids, petroleum products, and fertilizers to waters containing fish (Olmsted and Cloutman 1974, Meade 2004). These fish kills can be small in localized areas or extremely large, killing millions of fish. Point source additions of chemicals and/or nutrients have often been shown to result in fish kill events. However, point source pollution problems have been dramatically reduced since the turn of the 20th century, and most fish kills reported in the recent literature are due to natural events and mostly related to dissolved oxygen problems in nutrient-rich systems (Barica, 1975, Trim and Marcus, 1990; Townsend *et al.* 1992, Mhlange *et al.* 2006). If, however, the nutrient-rich condition is a consequence of anthropogenic activities, the term natural would not be appropriate. When a fish kill occurs, whether the result of natural or other causes, the public becomes greatly concerned. By the time anyone arrives at the scene of a fish kill, the water chemistry and/or chemical that caused the kill has generally changed or moved downstream. For this reason several researchers have attempted to develop models to predict the occurrence of fish kills in lake systems so management agencies can prepare for these events (Barica, 1975; Mericas and Malone, 1984; Miranda *et al.* 2001, Quinlan *et al.* 2005).

In order to have an insight in to the basic limnology of the water body and identify the causes for this unfortunate phenomenon, a study was conducted by the present authors starting 6th August, 2012 to check the physico-chemical features of the affected areas. As many authors have reported on the role of cyanobacteria

MATERIAL AND METHODS

Nigeen (Fig. 1) is one of the five basins of the world famous Dal Lake. It is situated at a distance of about nine kilometers to the north of Srinagar city India, at an elevation of 1584m a.s.l., covering an area of 4.5 km². The water supply of the basin is maintained by Hazratbal basin of the Dal Lake in addition to the springs within the basin, and atmospheric precipitation. The agricultural runoff and domestic effluents are the other sources of water supply.

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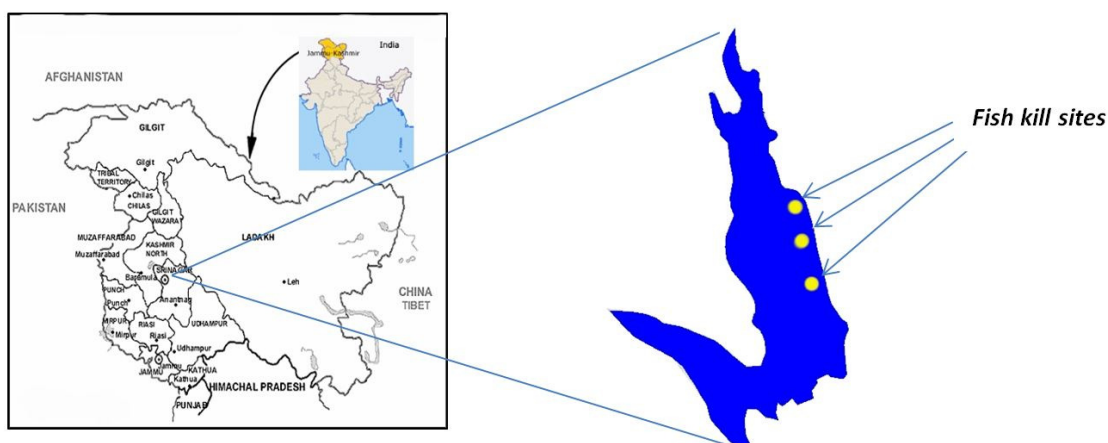


FIG.1: Location of Study Sites in Nigeen Basin

For determining the physio-chemical features, water samples from the fish kill zone were collected in one liter - plastic bottles. For determination of dissolved oxygen, DO bottles (glass) of 300 ml capacity were used. Water samples were analyzed in

the laboratory adopting standard methods given in Mackereth (1963) and APHA (2005) as explained in Table 1.

For Cyanobacterial identification, sample was gently shaken to re-suspend all materials. It was allowed to settle for one minute and then 1-2 drops were removed from the middle of the sample and placed on a glass slide. Identification of cyanobacteria was done with the help an Olympus phase-contrast microscope at 100 to 400X using taxonomic keys by Bellinger and Sigee (2010), Biggs (2000), Cox (1996) and Edmondson (1959). The quantitative estimation was done in a Sedgwick-Rafter chamber (S-R cell) under the same microscope. Each colony of *Microcystis* and *Anabaena* was counted as a single cell.

Table1. Water quality parameters, units and analytical methods used for water quality analysis.

S.No.	Parameters	Units	Analytical methods
1	Temperature	°C	<i>Instrumental method</i>
2	pH	pH units	<i>Potentiometric method</i>
3	Dissolved oxygen	mg L ⁻¹	<i>Winkler Azide -Modification method</i>
4	Electrical conductivity	μScm ⁻¹	<i>Conductivity cell potentiometric method</i>
5	Total alkalinity	mg L ⁻¹	<i>Titrimetric (methyl orange) method</i>
6	Chloride	mg L ⁻¹	<i>Argentometric method</i>
7	Total Hardness	mg L ⁻¹	<i>Complexometric method</i>
8	Total phosphorus	μg L ⁻¹	<i>Stannous chloride method</i>
9	Ammonia nitrogen	μg L ⁻¹	<i>Phenate spectrophotometric method</i>
10	Nitrate nitrogen	μg L ⁻¹	<i>Salicylate method</i>

RESULTS

The fish kill in Nigeen was characterized by three specific events that occurred during first week of August: Change in water quality/increase in water temperature, summer kill phenomenon and emergence of Cyanobacterial bloom. There were no recorded fish kill during the month of July which acted as our reference state. For the Comparative physicochemical analysis, water quality data prior (18th July) and after fish kill (6th August, i-e one day after fish kill) were studied (Table 2). Dissolved oxygen was found to be significantly ($p = 0.001$) lower in the range value of 0.4-1.6 mg/l and with mean value of 1.0 mg/l in the month of august (fish kill event) as compared to the July (no fish kill) with the mean value of 3.6 mg/l. Significant ($p < 0.05$) concentration of chloride was also observed after fish kill event with a mean value of 24.6 mg/l and on the other hand its mean value was 15 mg/l prior to the fish kill.

The lake tended to be alkaline at the time of fish kill with significantly high mean pH and mean alkalinities of 8 and 256 mg/l respectively. The lake was mostly productive after fish kill event with total phosphorus, Ammonia and Secchi depth averaging 66 $\mu\text{g/L}$, 280 $\mu\text{g/L}$ and 1.5 m, respectively. Water was highly turbid reducing the visibility of the lake.

Table 2. Mean value of eleven water chemistry variables measured in the Nigeen Lake before and after the fish kill event.

Parameter	<i>After fish kill</i>	<i>Before fish kill</i>
	Mean value	Mean value
Dissolved oxygen (mg/l)	1.0	3.6
Secchi depth (m)	1.5	2.5
Electrical conductivity (μScm^{-1})	400	180
Water Temperature ($^{\circ}\text{C}$)	29.5	25
pH	8.0	7.32
Chloride(mg/l)	24.6	15
Alkalinity(mg/l)	256	94
T. Hardness (mg/l)	130	88
Nitrate ($\mu\text{g/l}$)	303	121
T. Phosphorus ($\mu\text{g/l}$)	66	57
Ammonia ($\mu\text{g/l}$)	280	90

The means were compared with an analysis of variance and parameters were significantly different ($p \leq 0.05$)

Thousands of dead fish, mostly *Crossocheilus diplocheilus* commonly known as *Tethur*, were observed on the shore. Some other fishes which were also affected by the fish kill included *Schizothorax* sp., *Cyprinus carpio* and *Puntius conchonius*. The dead fish were found to be accumulating in shallow water and along the shore. The densest quantity of dead fish was on the eastern side of the Nigeen. The amount of dead fish couldn't be quantified as the fish that remained on the bottom of the lake or that were consumed by scavengers was unknown. Upon examining the dead fish, we found some unique signs; (i) *Discoloration*, (ii) *reddening of the skin*, (iii) *black & white spots on the skin*, (iv) *Abnormal shape*, (v) *Swollen areas*, (vi) *Abnormal lumps*, (vii) *Bulged eyes (Popeyes)* (viii) *Lesions*.

Cyanobacteria were present in various patches in the Nigeen Lake mostly comprising *Microcystis* and *Anabaena* sp. (Fig. 2). They considerably reduced the light penetration in the Lake.

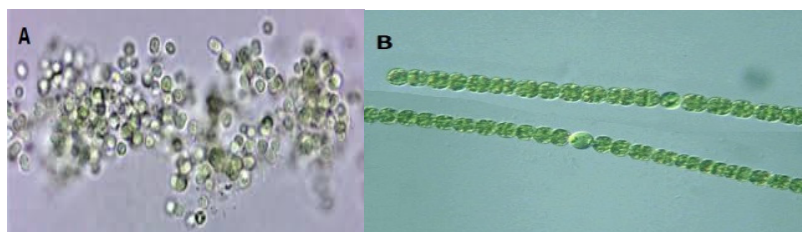


Fig. 2: Microscopic view of *Microcystis* sp.(A) and *Anabaena* sp.(B)

Nigeen Lake experienced what is called summer kill phenomena. Hot, cloudy and calm conditions prevailed for some days just before fish kill. Due to cloudy skies, photosynthetic rate was considerably reduced. As a result, primary producers (particularly algae) started diminishing, increasing the decomposing mass of the lake. The decomposing bacteria consumed the Lake's dissolved oxygen upon

decomposition, which put the fish under stress. During night, the process of photosynthesis stopped but the decomposition continued which drastically reduced the already weakened oxygen profile. This means that a mass fish kill had occurred at night or during the early morning hours.

Interestingly, drought like situation prevailed in the valley just before fish kill. In June and July, the average rainfall in the Valley used to be 95 mm, while as in summer 2012; it was just 46 mm affecting the eco-system of water bodies. Hot (up to 34°C) and cloudy weather was prevalent before the fish kill. The nearly two-month long dry spell was finally broken by sudden and intense rainfall. Soon after the rainfall, mass mortality of fish was observed.

DISCUSSION

Nigeen basin is the narrow stretch of water making it ideal place for stationing house boats and conducting aquatic sports as a result the basin has been tremendously stressed. When fish kill occurred, the first assumption was that something terrible wrong was with the water body. Suspicions were raised as to whether human activity, such as a chemical spill, may have caused the fish to die. Sometimes these suspicions are warranted but most times they are not.

Three mechanisms were found to play their role synergistically in the massive fish kill, summer kill phenomena, Change in water quality/water temperature, and Emergence of Cyanobacterial bloom.

Although oxygen depletions can happen at any time, they are most likely to occur during warm summer months. A combination of hot weather and cloudy skies can be particularly deadly for fish, as the decrease in sunlight (i.e., from cloud cover) makes it difficult for algae and plants to photosynthesize. The reduction in photosynthesis results in a decrease in oxygen being released into the water column. When overcast skies persist for several days, oxygen levels can become severely depleted. (Florida LAKEWATCH, 2003). This is exactly what happened in the Nigeen Lake.

The time of year a fish kill occurs can help in the determination of potential causes for the kill, and early on researchers reported observations that many fish kills occur in the summer after storm events (Swingle, 1968 and Barica, 1975). The examination of fish kill in Nigeen Lake strongly supports both observations. The fish kill in Nigeen Lake occurred in the warm month of August, and the frequency was highly correlated to the amount of rainfall and the elevated temperature of the water. Heavy thunderstorms can also have an adverse effect on oxygen levels, especially after extended periods of dry weather or during hot weather. If conditions have been dry for a long time, heavy rains tend to wash large amounts of organic matter such as dried leaves, grasses, etc. into nearby canals, lakes, and ponds. As bacterial organisms begin to decompose the new material, oxygen is used at a faster rate than normal. This can be a problem during hot weather as there is less oxygen in the water. (Florida LAKEWATCH, 2003). Similar type of fish kill was also found in our study as the Lake body experienced continuous rainfall for two to three days after a long dry period. Strong thunderstorms with abundant rainfall can dramatically change water chemistry of a lake in many ways; so several mechanisms probably work independently and/or in concert to produce a fish kill. Townsend *et al.* (1992) documented a fish kill in Donkey Camp Pool, Australia, caused by a large storm that increased an organic load with a high oxygen demand. Thus, while fish kill frequency is strongly correlated to rainfall events, the exact mechanisms that cause individual fish kills are probably different and lake dependent. Systematically investigating fish kills will most often yield information needed to determine the exact mechanism of individual fish kills (USFWS, 1990; AFS, 1992).

Lakes in summer are subject to much higher surface temperatures, decreasing the physical ability of water to hold oxygen. Many lakes are also stratified during the summer making it difficult for oxygen to diffuse to greater depths. Data from Nigeen Lake showed that it had the highest algal abundance in summer between June and October. Given these factors, the more biological activity in a lake, the greater the chances some climatic event will switch an oxygen balance to more respiration than

photosynthesis. From the water chemistry of the Nigeen Lake it is evident that more productive eutrophic lakes have a higher probability of experiencing a fish kill; therefore, any summer period natural or human-induced limnological change in these productive lakes that causes algal or aquatic plant populations to collapse or respire more than photosynthesize has the potential to cause a fish kill.

Our results suggest that the excessive fish kill in Nigeen Lake in August was mainly caused by the low dissolved oxygen content in the water during night accompanied with high temperature as well as high pH and ammonia. Warm water is less capable of holding oxygen gas in solution than cool water. This physical phenomenon puts the fish in double jeopardy because at high water temperatures their metabolic rates increase, hence their physiologic demand for oxygen increases (Francis-Floyd, 2003). Most species of fish are distressed when the oxygen content falls to $2\text{--}4\text{ mgL}^{-1}$ (Muller and Stadelmann, 2004); mortality usually occurs at concentrations less than 2 mgL^{-1} (Francis-Floyd, 2003). The ability to tolerate low dissolved oxygen levels depends on the species of fish (Francis-Floyd, 2003). *Crossocheilus diplochilus* had suffered the most in Nigeen Lake.

The increase in water temperature was accompanied by sudden changes in water pH and ammonia (Table 2). At elevated levels of pH, most of the ammonium is converted to toxic ammonia (NH_3), which can kill fish (Randall and Wicks, 2000). According to Randall & Wicks (2000), many fishes have difficulty excreting ammonia when exposed to alkaline conditions. As a result toxic levels of ammonia may rise in the fish due to impaired excretion, which may prove fatal to the fish.

Oxygen depletion in eutrophic lakes at night is a well-known consequence of “excess” algal biomass. Mass mortality of fish may be associated with the bloom of *Microcystis* and *Anabaena*. Supporting our hypothesis can also be drawn from other studies (Schwimmer and Schwimmer, 1964; Collins, 1978) which suggest that a critical masses of cyanobacteria decomposed naturally and this decomposed products plus toxic cellular materials released into the water during cell lysis might have caused death of fishes. Cyanobacteria can produce a wide range of toxins,

including microcystins (Tanner *et al.*, 2005). The concentration of these toxins may not be lethal to the fish, but they may affect the condition of the fish, making them more vulnerable to the unfavorable environmental conditions (Wolfstein, 2003). The likelihood of appearance of ecologically critical situations in lake depends on weather conditions. In Nigeen Lake, extensive fish kills are likely to occur again in summers (as happened in August, 2013), when high temperature is accompanied with low dissolved oxygen. Eutrophication and increase in temperature are acting in the same direction: both are increasing the probability of fish kill.

Our findings are consistent with decades of conventional wisdom in the primary literature and centuries of aquaculture practices. Fish kill generally occur in nutrient-rich systems, in the summer during the hottest months. These kills can be triggered by storms and increased rainfall that also coincide with the summer period. We recommend that state agencies (Fisheries department and LAWDA) in charge of fish populations use resources to systematically investigate all fish kills and maintain reports that can later be merged with ambient limnological data. Analyses like these will help agencies better understand and potentially predict the occurrences of fish kills.

CONCLUSION

Many different mechanisms working independently or in concert can be responsible for a fish kill, depending on the limnology of an individual water body. In summer, high temperature spells the high oxidation of nutrients and brisk depletion of oxygen levels in the lake can lead to such a phenomenon.

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Plant Coumarins - Occurrence, Biosynthesis and Perspectives

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ABSTRACT

Natural products deal with the isolation, identification, structure elucidation, and study of the chemical characteristics of chemical substances produced by living organisms. Coumarins are one of important class of natural products. This review highlights their occurrence, biosynthesis and perspectives.

Keywords: Plant Coumarins, occurrence, biosynthesis, perspectives

INTRODUCTION

Coumarins are derived from 1,2-benzopyrones. These molecules are found in higher plants where they originate from the general phenylpropanoid pathway (Harborne 1999) and are subject to numerous modifications. Coumarins continue to receive attention for their diverse bioactivities. Some natural coumarins have been used as human therapeutics, while 4-hydroxycoumarins are prominent examples of microbial modification which gave rise to the first generation molecules developed along with aspirin and heparin as anticoagulants (Mueller, 2004). Other applications appear possible in the course of new developments in various therapeutic fields, like symptomatic treatment of multiple sclerosis, photochemotherapy of T cell lymphoma, chemotherapy of multidrug resistant tumors, organ transplants, or treatment of smokers for nicotine addiction. Despite the importance of coumarins for

plant life and human uses, major details of their biosynthesis have remained unresolved. This review will give an update of coumarin biogenesis in plants with emphasis on the cytochrome P450 enzymes involved.

Coumarin (Fig. 1) is a natural product well known for its pleasant vanilla-like odor. It was reported from many plants of a variety of families, including Fabaceae i.e., Tonka bean (*Coumarouna odorata*) or sweetclover (*Melilotus alba*), Lamiaceae i.e., lavender (*Lavandula officinalis*), and Lauraceae i.e., cinnamon (*Cinnamomum verum*). More recent studies have revealed the presence of *o*-coumaric acid in *Arabidopsis thaliana* root exudates. As *cis-o*-coumaric acid is unstable under acidic or neutral conditions and lactonizes spontaneously to coumarin it is conceivable that coumarin is formed in *Arabidopsis thaliana*. There have been many reports on the effect of coumarin in plants, at the organ, tissue and cellular levels. These observations tend to demonstrate that coumarin acts as a plant hormone. However, until now neither solid evidence for a physiological function nor the molecular mode of action of coumarin has been provided in plant tissues.

2. Occurrence and functions of coumarins in plants

Coumarins may be sub classified as simple coumarins (benzo- α -pyrones syn. 1,2-benzopyrone), 7-oxygenated coumarins (furanocoumarins syn. furobenzo- α -pyrones or furocoumarins), pyranocoumarins (benzodipyran-2-ones), and phenylcoumarins (benzo-benzopyrones. Simple coumarins, furanocoumarins and pyranocoumarins derive from the same pathway, whereas the most common phenylcoumarins (i.e., coumestans) originate from isoflavone metabolism.

2.1. Simple Coumarins

These compounds are widespread in plants and more than 700 structures have already been described (Harborne, 1999). Coumarins formed by plants originate via shikimic and chorismic acids as well as phenylalanine and cinnamic acids with carbon dioxide being the ultimate source. The formation of the phenylpropanoid amino acids

phenylalanine and tyrosine via this pathway and the conversion of former to trans-cinnamic acid through the action of phenylalanine ammonia-lyase. The first step in the biosynthesis of the coumarin nucleus involves *ortho*-hydroxylation of cinnamic acid. Brown (Brown *et al.*, 1966) proposed the basis of tracer investigation that *trans*-cinnamic acid is the common precursor of all coumarins and that the *ortho* or *para* hydroxylation leads to the elaboration of coumarin or the 7-hydroxycoumarin.

Very early in studies on coumarin biosynthesis the *trans* isomer of cinnamic acid, *trans*-2'-glucosyloxycinnamic acid and its aglycone were implicated. Kinetic studies after administration of ^{14}C provided definite choice that *trans*-2'-glucosyloxycinnamic acid is an intermediate in the formation of coumarin. Glucosylation of *trans*-2'-hydroxycinnamic acid has been demonstrated in cell free extracts of *Morus alba*.

2.2. Hydroxylated and methoxylated coumarins

Prevalent hydroxylated coumarins are umbelliferone, herniarin and scoparone (2-methoxylated derivatives of umbelliferone), esculetin, fraxetin, isofraxidin, isoscopoletin, daphnetin and their corresponding glucosides (Fig. 1). As for scopoletin, these molecules are involved in plant responses to stressors like salicylic acid. Herniarin was demonstrated to be demethylated to umbelliferone by C4H from *Helianthus tuberosus* (CYP73A1) heterologously expressed in yeast; However, the K_m was so high compared to cinnamate substrate that the implication of C4H for herniarin demethylation remains questionable.

Scopoletin and scopolin (7- β - D -glucoside of scopoletin, Fig.1) were reported from many plants, e.g., rubber tree and cassava or carrot and cotton, but have been mainly studied in tobacco and sunflower. Scopoletin is a typical phytoalexin, its synthesis is post-infectionally activated in plants, but can also be triggered by various abiotic stresses. Scopoletin also displays radical scavenging properties toward reactive oxygen species and may be involved in the reduction of oxidative stress in plant cells. Until recently, there was no report of hydroxylated coumarins in *Arabidopsis*, however, recent metabolic studies have revealed that this plant can accumulate scopolin in

stems and roots. These findings demonstrate that stress induced hydroxylated coumarins are more common in higher plant species than previously assumed. As frequently described for other secondary metabolites (Harborne 1999), scopoletin is glucosylated to scopolin (Fig. 1) in the cytosol and then transferred to the vacuole.

Derivatives of daphnetin have attracted most attention recently. Cold acclimated rye expresses an *O*-methyltransferase with attenuated specificity for position 8. The product, 7-hydroxy-8-methoxycoumarin (hydrangetin) (Fig. 1), had been reported as a protein kinase inhibitor, and the modulating effect on protein kinases was proposed to function during exposure of rye to high photosystem II excitation pressure and cold acclimation. This might be the first example of a coumarin involved in hormone-like signaling. Polyhydroxylated coumarins, like 6,7,8-trihydroxycoumarin, have been described from *Pelargonium sinoides*, which demonstrates that plants are capable of multiple-step hydroxylations leading to more complex coumarin patterns.

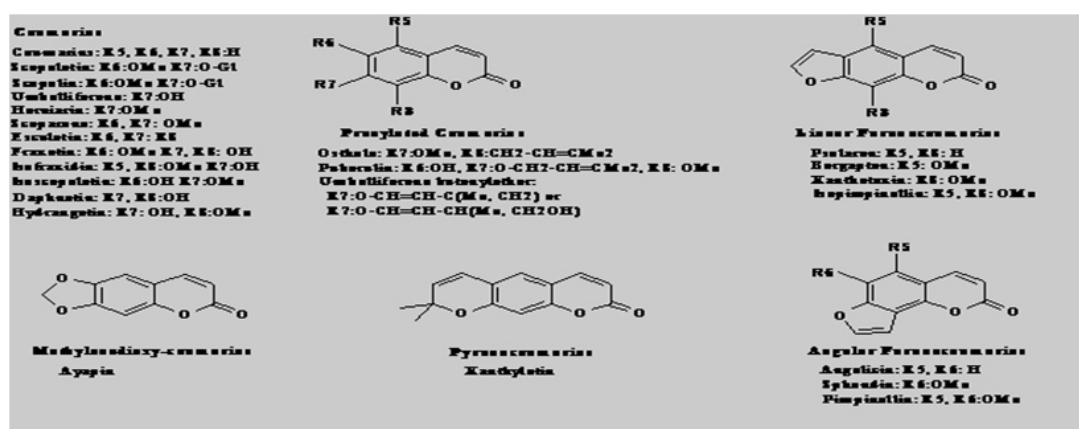


Fig. 1 Types of coumarins found in higher plants

2.3. Minor coumarins

There have been reports on many other minor coumarins in the phytochemical literature, which are beyond the scope of this review. Amongst this vast chemical diversity, methylenedioxy-substituted coumarins, i.e., ayapin (Fig. 1), and prenylated coumarins, like osthole and puberulin (Fig. 1), deserve mentioning. Ayapin has been described from Asteraceae only and was characterized as a phytoalexin. Methylenedioxy bridge-formation commonly occurs through cyclization of an ortho-methoxyphenol and is catalyzed by cytochrome P450-dependent activities. Such compounds are difficult to detoxify by phytopathogenic fungi, and it is noteworthy that the methylenedioxy moiety is known as a potent P450 inhibitor group requiring bioactivation. Osthole and puberulin have been frequently reported from Rutaceae and Apiaceae. *O*-Prenylated coumarins may be desaturated further to the corresponding butenyl ethers (Fig. 1) as shown in *Ammi majus*, and these reactions are likely also catalyzed through P450 enzymes. The butenylethers are labile and release a potentially toxic aldehyde moiety, which contributes to their role as phytoalexins. Thus, the aliphatic substitution of umbelliferone may provide new substrates for further cytochrome P450 modifications, but neither of these enzymes has so far been identified. As in case of ayapin in Asteraceae, the P450 monooxygenases must be considered as essential ecological factors.

2.4. Furanocoumarins

Furanocoumarins can be grouped into the linear type, where the (dihydro)furan ring is attached at C(6) and C(7), and the angular type, carrying the substitution at C(7) and C(8). Linear furocoumarins (syn. psoralens) are principally distributed in four angiosperm families: Apiaceae, Moraceae, Rutaceae and Leguminosae (restricted to Psoralea and Coronilla genera). The angular (dihydro)furanocoumarins are less widely distributed and primarily confined to the Apiaceae and Leguminosae. The most

abundant linear furanocoumarins are psoralen, xanthotoxin, bergapten and isopimpinellin, whereas the angular type is mostly represented by angelicin, sphondin, and pimpinellin (Fig 1). As was mentioned for the simple coumarins, numerous minor furocoumarins have been described in the literature, like bergamottin (5-geranoxypsoralen) which has received attention recently as a major grapefruit component interfering with drug metabolism by intestinal CYP3A4. Furanocoumarins are recognized as potent phytoalexins and allelochemical compounds. An outstanding feature of linear furanocoumarins is their ability to intercalate into dsDNA and create covalent cross-links primarily with thymidine residues. Cross linking proceeds readily under photoactivation and potentially blocks DNA replication and transcription. Accordingly, psoralens exhibit strong genotoxicity toward all living organisms, whereas the angular furanocoumarins are just capable of forming mono-adducts with DNA creating much less damage. Another remarkable property of furanocoumarins is their reactivity to inactivate P450 enzymes as mentioned above for bergamottin. This kind of enzyme inhibition has been demonstrated for P450s from vertebrate, insect and plant sources (Gravot et al. 2004). Psoralens inactivate by a mechanism-based inhibition (also referred as suicide inhibition) which requires their conversion to reactive intermediates by the enzyme itself. These intermediates form covalent links to the apoprotein and permanently inactivate the enzyme. The reactivity of furanocoumarins bears considerable ecotoxicological consequences, i.e., attributing these compounds an important role as allelochemicals during plant-insect interactions. Only herbivores able to tolerate furanocoumarins can feed on psoralen-rich plants, and xanthotoxin-insensitive P450 forms have been described from *Papilio polyxenes*, a papilionid butterfly adapted to furanocoumarin-accumulating host plants. This insensitivity was supposed to be the result of coevolution of insect detoxifying enzymes and the particular phytochemical defense since *Papilio glaucus*-whose host-plants do not contain furanocoumarins- exhibits sensitive P450s. The race of coevolution of butterflies on Apiaceae host plants has been studied in detail. The capacity of *Papilio*

polyxenes to detoxify furanocoumarins through CYP6B1 follows the order xanthotoxin > psoralen > angelicin (Wen et al. 2003), but a synergistic effect has been described between angular furanocoumarins and psoralen or xanthotoxin in response to insect attack. Considering the minor direct toxicity of angular furanocoumarins, the synergism is conceivably based on the inhibition of psoralen detoxifying CYP by angelicin. Furthermore, the accumulation of angular furanocoumarins is confined to a few taxons only. It was hypothesized, therefore, that the capacity for angular furanocoumarin biosynthesis has evolved later and presumably as a consequence to compensate for the success of herbivores in the detoxification of psoralens. It remains to be established, whether the enzymes for angular furanocoumarin biosynthesis have evolved from the biosynthesis of linear furanocoumarins. Most plants accumulating furanocoumarins possess a highly inducible biosynthetic pathway, which can be triggered by various biotic and abiotic stresses. *Ruta graveolens*, and possibly other Rutaceae, are exceptional because they do not respond to stressors and synthesize constitutively furanocoumarins in all tissues. However, the elicitation is still possible in *Ruta graveolens* dedifferentiated cells. The tissue-specific distribution of furanocoumarins has been studied in Apiaceae and Rutaceae. Obviously, these compounds accumulate in cells as well on the surface of plants. The pronounced accumulation on seeds and reproductive organs matches the optimal defense theory which predicts that defense compounds are principally allocated to the organs that play a key-role in plant fitness. The sub cellular localization of furanocoumarins is still unknown, but glucosylated forms have been frequently reported, suggesting a probable vacuolar compartmentation.

2.5. Pyranocoumarins

Pyranocoumarins, like xanthyletin (Fig. 1), have been mainly described from. As for furanocoumarins, linear and angular forms can be distinguished. To our knowledge, there is no proposal on their functions in plants, however, due to the structural

relationship with furanocoumarins, a role as phytoalexins may be assumed. The biosynthesis of pyranocoumarins has not yet been investigated.

3. Biosynthesis of coumarins in plants

Main enzymes and genes implicated in coumarins biosynthesis and that have been sufficiently documented.

3.1. Cinnamic acid to coumarin

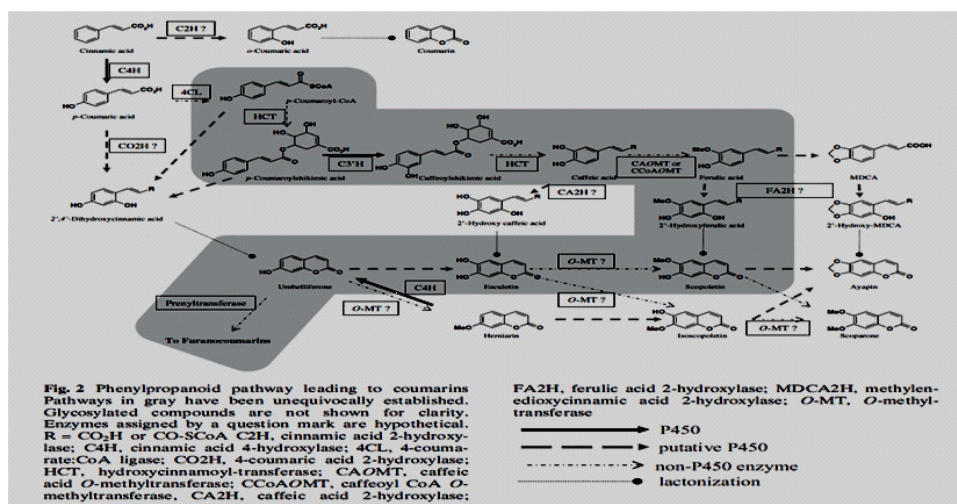
The pathway of coumarin biosynthesis has been largely outlined during the '60s and '70s, with the help of tracer feeding experiments. Radiolabeled cinnamic acid was incorporated into coumarin and 7-hydroxycoumarins. Other tracer experiments conducted with *Lavandula officinalis*, a plant that produces coumarin as well as 7-hydroxylated coumarins, revealed that in the latter instance *para*-hydroxylation preceded the *ortho*-hydroxylation required for lactonization. This indicated that umbelliferone (Fig. 1) is derived from *cis-p*-coumaric acid, whereas coumarin originates from *cis*-cinnamic acid (Fig. 2), and may imply different enzymes for the *ortho*-hydroxylation/lactonization of coumarin versus umbelliferone.

The *ortho*-hydroxylation is a key step of coumarin biosynthesis, that has received insufficient attention. In initial experiments, double-labeled (*ortho*-³H, *ring*-1-¹⁴C) cinnamic acid was fed to *Melilotus alba* shoots or *Gaultheria procumbens* leaves, and the retention of label was monitored upon conversion to *o*-coumaric acid. An NIH shift was proposed because of insignificant decrease of the ³H:¹⁴C ratio, which is an indication of a cytochrome P450 monooxygenase reaction mechanism. A following report addressed the formation of coumarin with extracts from *Melilotus alba*, a plant that produces high levels of coumarin. This study allocated the *ortho*-hydroxylation of cinnamic acid to the chloroplast and again suggested a P450-dependent hydroxylation mechanism. Unfortunately, the *in vitro* results could not be reproduced, and the class of the enzyme involved as well as its subcellular site remain to be established. As revealed later, the early experiments may have suffered from fundamental analytical problems,

since the chromatography and recrystallization techniques employed were likely insufficient to separate the various cinnamic acids. Nevertheless, the proposed conversion of cinnamic to *o*-coumaric acid received some support by precursor feeding studies done with *Petunia* chloroplasts, which ascribed cinnamate 2-hydroxylase, including the formation of coumarin, and lack of cinnamate 4-hydroxylase to these organelles. In light of the studies done since with *Ammi majus* microsomes on the biosynthesis of furanocoumarins it appears possible that the 'ortho-hydroxylase' is an exceptionally labile CYP enzyme, in contrast to the CYPs hydroxylating cinnamic acids in *para* or *meta*- position (Fig. 2). Overall, the *ortho*-hydroxylation of cinnamic (or 4-coumaric) acid, being of pivotal importance for all coumarins, remains a missing link in the network of phenylpropanoid biosynthesis.

3.2. *Cinnamic acid to umbelliferone and other hydroxylated coumarins*

The formation of umbelliferone proceeds from 4-coumaric acid or its ester derivatives (Fig. 2). The conversion of cinnamic acid to 4-coumaric acid is catalyzed by cinnamate 4-hydroxylase, a cytochrome P450 monooxygenase from the CYP73A family. This enzyme constitutes the P450 enzyme most studied to date and sets the stage for several branch pathways, such as the lignification (Anterola and Lewis, 2002) or flavonoid biosynthesis (Harborne, 1999).



Following the pertaining literature, 4-coumaric acid is *ortho*-hydroxylated to 2,4-dihydroxycinnamic acid. The respective enzyme activity was reported exclusively from *Hydrangea macrophylla* and assigned to the chloroplasts. This enzyme fraction was demonstrated to slowly convert cinnamic acid to *o*-coumaric acid but was more active to transform *p*-coumaric acid and ferulic acid respectively to umbelliferone and scopoletin. Although this report is unique in describing the *ortho*-hydroxylation of a hydroxylated coumarin in vitro and suggesting one plastidic fraction for the *o*-hydroxylation of both *p*-coumaric and ferulic acid as well as benzoic acid, the conversion of ferulic acid to scopoletin had been postulated before from precursor feeding studies in tobacco tissue cultures. This biosynthetic course of scopoletin/scopolin has been recently established in *Arabidopsis thaliana*. T-DNA insertion mutants within the gene encoding CYP98A3, which catalyzes 3'-hydroxylation of *p*-coumarate, revealed a dramatic decrease in both scopoletin and scopolin contents, confirming the origin from ferulic acid in *Arabidopsis*. This is in contrast to the results obtained for puberulin (Fig. 1) biosynthesis in *Agathosma puberula*. Here, as well as in *Daphne mezereum*, ferulic acid was not readily

incorporated as opposed to umbelliferone, therefore making esculetin (Fig. 1) a likely precursor for the synthesis of scopoletin. The formation of esculetin (Fig. 1; 6,7-dihydroxy coumarin) was examined in *Cichorium intybus*. These studies revealed that umbelliferone was an efficient precursor but not caffeic acid, suggesting 6-hydroxylation of umbelliferone, probably by the action of a P450 monooxygenase. This deserves mentioning, because the conversion of caffeic acid to esculetin is readily accomplished in vitro with various plant extracts containing phenol oxidase activity, but has not been confirmed in plants. Similar to esculetin, daphnetin (Fig. 1, 7,8-dihydroxycoumarin) in *Daphne mezereum*, was shown to be derived from umbelliferone rather than caffeic acid.

3.3 The ortho-hydroxylation: a common route with salicylic acid

Analogous to C2H, another major *ortho*-hydroxylation step in phenolic metabolism is still controversial. Salicylic acid is a pivotal signal molecule in plant defense mechanisms but the biosynthesis pathway is still matter of debate. Two routes have been proposed. A pathway already shown to occur in bacteria has been proposed in tobacco through chorismate and isochorismate, via the general shikimic acid metabolism (Wildermuth *et al.*, 2001). Another route has been documented in tobacco and rice, via decarboxylation of transcinnamic acid to benzoic acid and subsequent 2-hydroxylation. This benzoic acid 2-hydroxylase was characterized as a P450 enzyme but important biochemical characteristics are atypical for an eucaryotic P450 as it appears to be soluble and it exhibits an unusually high molecular weight. The corresponding P450 gene has not been reported so far. This benzoic acid 2-hydroxylase is unable to transform cinnamic acid into *o*-coumaric acid and consequently is unlikely to interfere with the coumarin pathway.

3.4. Biosynthesis of furanocoumarins in plants

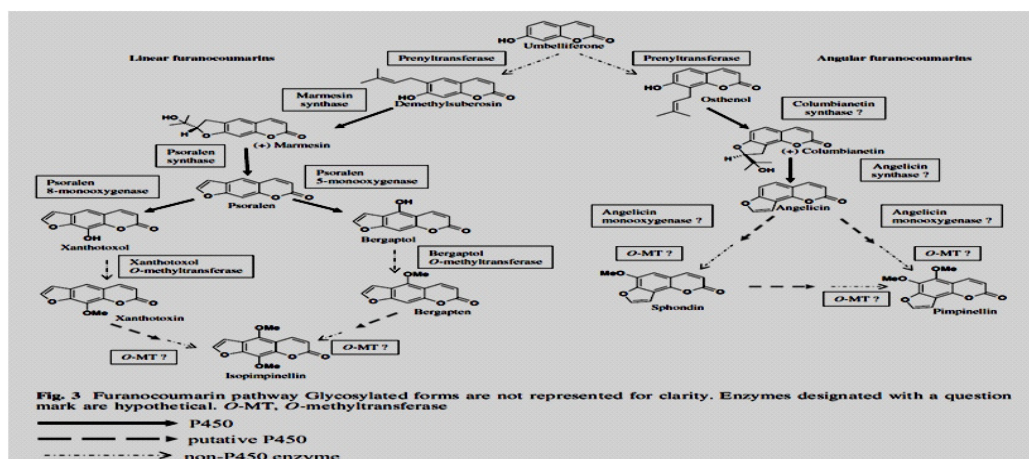
While coumarin biosynthesis remains a black box, several enzymes of the furanocoumarin pathway have been isolated and characterized (Fig. 3). Umbelliferone

rather than coumarin is the parent compound of furanocoumarins, as was reported a long time ago. It is first prenylated in 6- (for linear furanocoumarins) or 8-position (for angular furanocoumarins) to yield demethylsuberosin and osthenol, respectively (Fig. 3). Dimethylallyl diphosphate required for the 6-prenylation at least is provided in celery (*Apium graveolens*) by the deoxy-D-xylulose pathway and not through the mevalonate-dependent pathway. This is conceivably also the case in other plants, because the prenyltransferase has been identified in *Ruta graveolens* as a plastidic enzyme, and the activity was also documented in *Ammi majus*. The homologous enzyme for the angular furanocoumarins has not been isolated so far.

3.4.1 Linear furanocoumarins

Demethylsuberosin is transformed to marmesin and further to psoralen by two separate cytochrome P450 enzymes. The enzymes were biochemically characterized, and evidence for their P450 nature was obtained from characteristic blue-light-reversible inhibition of the activities by carbon monoxide, and the use of specific inhibitors. The two enzymes formally catalyze very different reactions, the first forming the dihydrofuran-ring from the *ortho*-prenylated phenol (marmesin synthase) and the second catalyzing the oxidative carbon-carbon chain cleavage reaction (psoralen synthase). The mechanism of marmesin synthase has not been solved yet, but it might be speculated that some analogy exists to menthofuran synthase from *Mentha piperita* which belongs to the CYP71 family (Croteau et al., 2005). Psoralen synthase was found to operate by syn-elimination of acetone and one hydrogen from position 3' (Fig. 3). This release of acetone is unique in plants. Psoralen synthase is very specific for (+)-marmesin and does not accept the (-)-stereoisomer (nodakenetin) as a substrate. Neither of the two P450s has been characterized at the gene level.

3.4.1 Linear furanocoumarins



Psoralen 5-monooxygenase catalyzes the subsequent hydroxylation of psoralen to bergaptol and was also characterized as a cytochrome P450 enzyme from *Ammi majus* cell suspensions. Nevertheless, there is still the possibility that bergaptol could be formed from 5-hydroxymarmesin. Different plants might thus have developed a slightly different sequences. Bergaptol is then O-methylated to bergapten. The cDNA encoding the O-methyltransferase catalyzing this reaction was recently cloned and functionally characterized from *Ammi majus*. The enzyme was shown to be highly specific for bergaptol and does not accept xanthotoxol, the C(8) corresponding phenol. This corroborates previous reports on the separation of bergaptol and xanthotoxol methyltransferases from *Ruta graveolens* or *Petroselinum crispum*. The path for isopimpinellin formation (5, 8-dimethoxypsoralen) is uncertain. It was studied in *Heracleum lanatum*. In this plant xanthotoxin was the most efficient precursor. However, bergapten was found to be converted into isopimpinellin, although at a lower rate. Both 5- and 8-hydroxylation pathways can thus lead to final product, but 5,8-dihydroxypsoralen was also demonstrated to be a possible precursor in *Ruta graveolens*. Enzymatic turnover of the pathways could simply explain the prevalence of one of the three routes in a given plant.

3.4.2. Angular furanocoumarins

The transformation of columbianetin to angelicin is very similar from a mechanistic and stereochemical point of view to the conversion of marmesin to psoralen. As demonstrated by feeding studies using deuterium-labeled columbianetin with plants or leaf tissues. It is, thus, conceivable that the enzymes for angular furanocoumarin biosynthesis may have emerged by evolutionary adaptation from the linear pathway. This would be consistent with the fact that angular furanocoumarins are less abundant in plants than the linear type and that angular furanocoumarins are always found concomitantly with linear furanocoumarins. This hypothesis will be investigated once the genes for marmesin synthase and psoralen synthase, as well as those for umbelliferone 6- and 8-prenyltransferases, will be identified (Fig. 3). Unfortunately, no information is available yet at the genetic level.

3.4.3. Implication of P450s in furanocoumarin synthesis

Cytochrome P450 enzymes are pivotal enzymes of furanocoumarin biosynthesis, i.e., the formation of xanthotoxin relies, at least, on four sequential P450 reactions catalyzed by C4H, marmesin synthase, psoralen synthase and psoralen 8-monooxygenase. This was at a first glance puzzling because of the intrinsic capacity of furanocoumarins to inhibit very different cytochrome P450 enzymes, irrespective of the species, through a mechanism-based inactivation process. To understand how plants cope with this problem Gravot and co-workers compared inactivation by furanocoumarins (Gravot et al., 2004) of three different C4H: one from a plant that does not contain furanocoumarins (*Helianthus tuberosus*, CYP73 A1) and two from plants that synthesize furanocoumarins (*Ruta graveolens*, CYP73A32; *Petroselinum crispum* CYP73A10). This would suggest that plants producing furanocoumarins have adapted their P450 enzyme repertoire to the need for reduced inactivation while retaining the high catalytic efficiency. It is reasonable to expect a similar adaptation of all the P450 enzymes in the same pathway.

The evolution toward furanocoumarin accumulation must have occurred under strong selection pressure, since the biocidal and enzyme inactivation properties of furanocoumarins appear to be lethal to plants unless quick adaptation can be accomplished. This pressure might have built up by the exposure to herbivores and the need for efficient antifeedant metabolites. This would be fully compatible with the scheme of furanocoumarins as allele-chemicals in the warfare with insects only adapted to hatch on furanocoumarin producing plants (Schuler and Berenbaum, 2003). It will be interesting to compare the cytochrome P450 families recruited for the synthesis of furanocoumarins in the plant and their detoxification in insects.

4. Perspectives

Although no monooxygenase of the furanocoumarin pathway has been characterized at the gene level, techniques such as differential display and RT-PCR strategies have been developed for P450s, which should be readily applicable to furanocoumarin pathway. Such techniques already led to the characterization of the C4H and C3'H in the relevant plants. Inducible systems are needed to differentiate and correlate the individual transcript abundances with product accumulation. Elicitor-treated *Ammi majus* cultures appear to qualify for this purpose. Numerous recent studies focused on the role of furanocoumarins as key allelochemicals, but the physiological relevance of coumarins reaches far beyond in the producing plants. This includes the potential role of simple coumarins as hormones and signaling molecules, which were shown in the past decade to be much more widespread in plant kingdom than previously assumed. More functional insight should be obtained once the mechanism, regulation of their biosynthesis and their subcellular localization will be known. Biosynthesis of L-phenylalanine proceeds in plastids while phenylalanine ammonia-lyase and C4H activities reside in the cytosol and endoplasmic reticulum. Subcellular localization of the pivotal *ortho*-hydroxylation of cinnamic or 4-coumaric acid, so far, remains unresolved. Investigation in *Ruta graveolens* assigned the subsequent 6-prenylation of

umbelliferone to plastidial membranes. Clarification of the localization of the 2-hydroxylation will be the further step to understand the physiological role of coumarins. It is probable that different routes to coumarins will be discovered to operate in plants, some of them might be confined to a taxonomic group. The formation of scopoletin is an example and derives either from esculetin or ferulic acid according to the plant species considered. It is currently unknown, whether the P450s involved in the furocoumarin pathway belong to a single family, as is the case with CYP71s in benzoxazine synthesis, or to multiple P450 families as shown for biosynthesis of cyanogenic glucosides (CYP71E1 and CYP79A1). In either case, the discovery of genes involved in coumarin synthesis will add another stage of complexity to the phenylpropanoid pathway. The recent detection of coumarin and hydroxylated coumarins in *Arabidopsis thaliana* have opened the way for new approaches. Metabolomics in conjunction with screening of mutant libraries is likely to reveal new players in the coumarin pathway.

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Scientific Documentation of Wound Healing Efficacy of Herbal Drugs - A Review

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ABSTRACT

Wound is simply a loss of cellular and functional continuity. Its healing is natural process as long as wound environment and health status of the individual is optimum. Delayed healing besides causing morbidity and loss of function of the particular part inflicts heavy economic loss to the individual. Early healing of a wound therefore has been the field of attraction for research workers. Various workers in past have tried number of wound healing agents to enhance the wound healing process. The demand for plant based medicines, health products, pharmaceuticals, food supplement, cosmetics etc are increasing in both developing and developed countries, due to the growing recognition that the natural products are non-toxic, have less side effects and easily available at affordable prices. These herbal drugs need to be standardized and their mode of action studied. Many herbal drugs have been proved to have wound healing efficacy and others are under trial nowadays. This review has therefore been an attempt to documents the scientific validation of proven herbs having wound healing efficacy.

Keywords: Herbal medicine, rhubarb, wound healing

INTRODUCTION

Wound healing has got high priority among body functions. Despite tremendous advancement, an effective wound management continues to be a challenge to the clinician. In addition to conventional allopathic medicines including antibiotics,

analgesics, anti-inflammatory drugs many medicinal plant preparations and biological dressing materials have been used to enhance the wound healing rate. Mostly the wounds are managed by topical medicaments, but action of many such common preparations are often discouraging because of ensuing destruction of leucocytes and other cellular elements of the wound (Tyagi and Singh, 1993). Common pathogens causing wound infection like *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumonia* have been reported to have developed resistance against the targeted antibiotics. Such antibiotics are also showing adverse effects to the individuals (Mertz and Ovigten, 1993). Use of medicinal plants or their parts, either in whole form, or their extracts seems thus indispensable ((Essawi and Shour, 2000). Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparation for therapy. Furthermore plants are considered more potent healers because they promote the repair mechanism in the natural way. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body (Kamboj, 2000). The demand of herbal drugs is increasing day by day in developed as well as developing countries because they are safer and well tolerated as compared to allopathic drugs (Rawat *et al.*, 2012).

However in most of the cases mode of action studies, standardization based on chemical and activity profile and their safety and stability has not been documented (Kamboj, 2000). This review is an attempt to document the clinical trial of many medicinal plants used specifically for wound enhancing purpose.

Wound healing

Wound healing is a complex and dynamic process with the wound environment changing with the changing health status of the individual. Wound healing involves continuous cell-cell and cell-matrix interactions that allow the process to proceed in three overlapping phases namely, inflammation (03 days), cellular proliferation (312 days) and remodeling (36 months) (Glynn, 1981; Clark, 1996; Martin, 1996). Healing is not complete until the disrupted surfaces are firmly knit by collagen (Buffoni *et al.*, 1993). The inflammatory phase prepares the area for healing and immobilizes the wound by causing it to swell and become painful, so that movement becomes restricted. At the tissue level, increased vascular permeability and the sequential migration of leukocytes into the extravascular space characterize inflammation (Cohen *et al.*, 1999). Migrating monocytes transform into macrophages as they migrate into the extravascular space in a process that is stimulated by chemotactic factors such as fibronectin, elastin derived from damaged matrix, complement components, enzymatically active thrombin, TGF- β , and serum factors (Fukai *et al.*, 1991). After activation, macrophages and neutrophils initiate cellular wound debridement by phagocytosing bacteria and foreign material (Newman *et al.*, 1982). Macrophages also initiate the development of granulation tissue and release a variety of proinflammatory cytokines and growth factors (Kondo and Ishida 2010). The angiogenic process becomes active from day 2 after wounding (Grotendorst, 1984). In the first week post acute injury three processes epithelialisation, wound contraction and collagen production occur simultaneously to achieve coalescence and closure. Within hours of injury, the release of EGF, TGF- α , and FGF act to stimulate epithelial cell migration and proliferation through the acts of reproduction and mitosis, resulting in the start of reepithelialization. The formation of granulation tissue starts at the wound space approximately 4 days after injury. Numerous new capillaries endow the new stroma with its granular appearance. Macrophages, fibroblasts and endothelial cells

move into the wound space at the same time. Macrophages not only augment inflammatory response but also promote angiogenesis. Neovascularization is essential for the synthesis, deposition and organization of new extracellular matrix (Konda and Ishida, 2010). The remodeling phase is most essential phase. The remodeling phase envisages replacement of granulation tissue with a frame work of collagen and elastic fibres with revascularization. The final outcome of all these events is repair of damaged tissues by formation of scar (Guo and DiPietro, 2010). A successful contraction results in a smaller wound that needs to be repaired by scar formation. A specialised cell called a myofibroblast is involved in this contraction process. Myofibroblasts attach to the skin margins, pull the entire epidermal layer inward, Control of wound contraction and scar formation at the time of wound formation is useful in order to control the direction of wound contraction and thus prevent distortion (Zitelli, 1987).

Historical background and current scenario of herbal drugs

The use of plants for healing purposes predates human history and forms the origin of much of the modern medicine. The earliest evidence of human's use of plant for healing dates back to the Neanderthal period (Winslow and Kroll, 1998). Many conventional drugs originated from plant sources. A century ago, most of the conventional effective drugs were plant based. The earliest recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years (Pal and Shukla, 2003). The classical Indian texts making mention about the use of herbal drugs include Rigveda, Atharvaveda, Charak Samhita and Sushruta Samhita. The herbal medicines / traditional medicaments have therefore been derived from rich traditions of ancient civilizations and scientific heritage (Kamboj, 2000). Traditional Chinese medicine has been used by Chinese people from ancient times. About 5000 traditional remedies, including mostly herbs are available in China. They account for approximately one fifth of the entire Chinese pharmaceutical market (Li, 2000). Many herbal remedies found their way from China into the Japanese systems of

traditional healing. Herbs native to Japan were classified in the first pharmacopoeia of Japanese traditional medicine in the ninth century (Saito, 2000). Ayurveda (Indian traditional medicine) is a medical system primarily practised in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment (Morgan, 2002).

Worldwide, herbal medicine received a boost when the WHO encouraged developing countries to use traditional plant medicine to fulfill needs unmet by modern systems (Winslow and Kroll, 1998). In one survey it was estimated that 39% of all 520 new approved drugs in 1983-1994 were natural products or derived from natural products (Cragg *et al.*, 1997) and 60-80% of antibacterial and anticancer drugs were derived from natural products (Harvey, 1999). The wide spread use of herbal medicine is not restricted to developing countries, as it has been estimated that 70% of all medical doctors in France and German regularly prescribe herbal medicine (Murray and Pizzorno, 2000). With the US Food & Drug Administration (FDA) relaxing guidelines for the sale of herbal supplement, the US market is booming with herbal products (Brevoort, 1998; Gottlieb, 2000). Americans paid an estimated US\$ 21.2 billion for services provided by alternative medicine practitioners (Eisenberg, *et al.*, 1998).

India is sitting on a gold mine of well-recorded and well-practiced knowledge of traditional herbal medicine (Sharma *et al.*, 2008). In India around 20,000 medicinal plant species have been recorded (Dev, 1997), but more than 500 traditional communities use about 800 plant species for curing different diseases (Kamboj, 2000). The annual turnover of the Indian herbal medicinal industry is about Rs. 2,300 crore as against the pharmaceutical industry's turnover of Rs. 14,500 crores with a growth rate of 15 percent (Krishnan, 1998).

Application of herbal drugs in wound healing

Herbal drugs have been used to evaluate their wound healing efficacy in different animal models ranging from laboratory animal to large animals. Even cellular models

have proved quite effective for the evaluation of wound healing efficacy of herbal drugs (Graham, 2004). However among animal models rabbits have proved most useful. Rabbits are significantly larger than the more commonly used laboratory animals such as mice and rats, but remain easy to handle and inexpensive (Ramos *et al.*, 2008). Different wound models ranging from partial thickness to full thickness wounds or incisional wounds to excisional wounds have been used (Asif *et al.*, 2007). The efficacy of drugs has been evaluated from different parameters including gross, histopathological, bacteriological and even biochemical evaluation of different enzymes (Kalirajan *et al.*, 2012; Gupta *et al.*, 2007).

Herbal drugs have been used for wound healing in either crude form or in the form of different extracts. Kirtiker and Basu (1935) used poultices of plant *Vernonia cinera* and roots of *Eriolsena quinquelocularis* for treating wounds and sores. They found positive effect in healing of the wounds. Deshpande and Pathak (1966) used fresh juice of *Jasmina auricularum* and found 20% hastening in wound healing and 35% increase in tensile strength between 6-9 days after local treatment of wounds. Herbal drugs have been found equally effective in suppurative conditions of wounds also. A herbal skin gel "Charmil" caused faster wound healing in the treatment of experimentally induced suppurative wounds in calves. The healing effect of the gel is attributed to its antimicrobial, fly repellent and larvicidal effect (Pradhan, 1995). Volatile oil obtained from *Allium sativum*, *Curcuma longa* and alcoholic extract of fresh roots of *Moringa pterygosperma* inhibit the growth of both Gram positive and Gram negative organisms (Chopra *et al.*, 1958). Aqueous extract of *Helianthus annus* plant has been found to have healing effect. Aseptic wounds of albino rats became dry and scab was formed after 36 hours of wounding when treated with 5% aqueous extract of the plant. The contraction was complete by 15th day (Deshpande *et al.*, 1965). Powder, alcoholic and chloroform extract ointment of *Adhatoda vasica* treated wounds showed an early fibroblastic proliferation, less inflammatory changes and early epithelialization of newly formed connective tissue as compared to controls. Histo-chemical observations

revealed more collagen, elastin and mucopolysaccharide in treated wounds than control wounds, while insignificant changes were seen in extent of formation of reticulin fibers (Bhargava *et al.*, 1986). Zama *et al.*, (1991) reported that the rate of healing was highest in wounds treated with alcoholic extracts of *Adhatoda vesica*. Similarly powder, alcoholic or chloroform extract ointment of *Annona squamosa* showed early and better wound healing effect. Among these alcoholic extract ointment was found the best. The percent healing and tensile strength, collagen and elastin contents gradually increased from 3rd to 30th day and was higher in wounds treated with ointment of alcoholic extract of *A. squamosa*. Maximum concentration of hexosamine was found on day 3rd in all treated wounds upto day 30th. Zinc contents showed significant and gradual increase from day 3rd to 30th day in powder and alcoholic extract ointment treated wounds (Bhargava *et al.*, 1988). Aqueous extract of *Triticum vulgare* is effective in promoting the tissue repair process by supporting wound repair process through epidermal regeneration. Extract aids cutaneous wound healing due to its mitogenic effect on fibroblasts, and endothelial cells and by promoting epidermal cell proliferation in damaged epidermal areas (Mastroianni *et al.*, 1998). Wound healing activities of the aqueous and methanol extracts of the roots of *Berberis lyceum* has been shown in rats using incision, excision and dead wound space models in rats. Application of both extracts yields increased epithelilization, wound contraction, skin breaking strength, tissue granulation, dry weight and hydroxyproline content and collagen formation. The methanol extract is more effective than the aqueous extract (Asif *et al.*, 2007). *Bergenia ciliata* rhizome possesses promising therapeutic potential and could be considered as potential source for drug development by pharmaceutical companies as its methanolic and aqueous extracts have been found to have antioxidant activity (Venkatdhari *et al.*, 2010). The antimicrobial activity of methanolic and aqueous extracts of the medicinal plant *Cocculus hirsutus* bestows it potential wound healing activity. The methanolic extract of the plant was found to be more

effective against *V. cholera* and *S. aureus* and in the mean time the aqueous extract was found to be more effective against *V. cholera* and *K. pneumoniae*. Both methanolic and aqueous extracts of this plant have no fungicidal activity against any fungal pathogen (Kalirajan *et al.*, 2012).

On clinical, pathological, histochemical and microbiological studies healing was found best with *M. chamomilla* lotion, followed by *M. chamomilla* ointment and *P. bistorta* ointment, *Nigella sativa* lotion, and *P. bistorta* ointment, with *S. fragalis* lotion the least (Ahmed *et al.*, 1995). *Aloe vera* influences the healing process by enhancing collagen turnover in wound tissue. It increases biosynthesis of collagen and activity of collagenase (collagen degradation). It also increases levels of lysyl oxidase which indicates increased crosslinking of newly synthesized collagen (Chithra *et al.*, 1988). Asiaticoside isolated from *Centella asiatica* when applied topically as 0.2% solution causes 56% increase in hydroxyproline, 57% increase in tensile strength and increases collagen contents and better epithelialization. Topical application of 0.4 % solution of asiaticoside has been also found effective in streptozotocin-diabetic rats (where healing was delayed) where it increased hydroxyproline content, tensile strength, collagen content and epithelialization. Its oral administration @ 1mg/kg dose in guinea pig punch wounds was also found active. It promotes angiogenesis in chick chorioallantois membrane model at 40 µg concentration, thus it has been concluded that it exhibits significant wound healing activity in normal as well as delayed wound healing models (Shukla *et al.*, 1999).

Methanol extract of *Leucas avandulaefolia* both in the form of ointment as well as injection in both excisional and incisional rat wound models showed significant wound contracting ability, reduction in closure time, increase in tensile strength and regeneration of tissues at the wound site (Kakali-Saha *et al.*, 1997). *Rhodiola imbricate* rhizome ethanol, rich in polyphenols improves rate of wound contraction and decreases time taken for epithelialization. The extract also increases cellular proliferation

and collagen synthesis at the wound site, which could be evidenced by increase in DNA, protein, hydroxyproline and hexamine contents (Gupta *et al.*, 2007). Ethanolic extract of *Morinda citrifolia* leaves has been found to have wound-healing activity in rats, using excision and dead space wound models. Ethanolic extract of *Morinda citrifolia* when given orally @ 150 mg kg⁻¹ day⁻¹ by mixing in drinking water exhibited 71% reduction in the wound area when compared to 57% in controls. Enhanced wound contraction, decreased epithelialization time, increased hydroxyproline content and histological characteristics suggest that this leaf extract has therapeutic benefits in wound healing (Nayak *et al.*, 2009).

Rheum emodi, in Kashmiri called *Pambh challan* is a household herb, believed to have many potential therapeutic characteristics including wound healing. However its evaluation as under cutaneous trial was conducted by Aakhoon (2001). He evaluated its wound healing efficacy in cow calves and credited it with anti-inflammatory, anti-bacterial and wound healing properties. Tang *et al.*, (2007) used emodin derived from Rhubarb topically and recorded increased content of hydroxyproline on day 7 post-wounding and more tensile strength on day 14 post-wounding in treated wounds. The mechanism attributed to the healing efficacy of *Rheum sp* include a complex mechanism involving stimulation of tissue regeneration and regulating Smads-mediated TGF-beta signaling pathway. Ethyl acetate extract of *R. emodi* has been reported having an immune-enhancing effect leading to anti-tumor, wound healing and antibacterial effects via Th-1 and Th-2 cytokine regulation in vivo and reported that the molecular mechanism involved is attributed to the increase in the release of NO, IL-12 and TNF- α and a decrease in the production of IL-10 as observed with RAW 264.7 cell lines (Kousar *et al.*, 2011).

Safety and health concerns

Safety is utmost important provision of herbal medicines and herbal products for health care, and a critical component of quality control. Over the past decade, interest

in drugs derived from higher plants has increased expressively. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants (Farnsworth and Morris, 1976; De Smet, 1997; Cragg *et al.*, 1997; Shu, 1998). Among consumers, there is a widespread misconception that “natural” always means “safe”, and a common belief that remedies from natural origin are harmless and carry no risk. However, some medicinal plants are inherently toxic. Plants have hundreds of constituents and some are very toxic such as the most cytotoxic anti-cancer plant-derived drugs, digitalis, the pyrrolizidine alkaloids, ephedrine, phorbol esters, etc. However, the adverse effects of most herbal drugs are relatively less frequent when the drugs are used properly compared with synthetic drugs, but well-controlled clinical trials now confirm that they really exist (Bagheri *et al.*, 1998; Brinker, 1998; Brown, 1992; D'Arcy 1993; De Smet, 1995; Farnsworth, 1993; Drew & Myers, 1997). Most herbal products in the market today have not been subjected to drug approval process to demonstrate their safety and effectiveness. Some of them contain mercury, lead, arsenic (Kew *et al.*, 1993), corticosteroids and poisonous organic substances in harmful amount (De Smet, 1997). Sometimes patients use traditional and conventional medicines simultaneously. The interaction of these two types of drugs in vivo may be dangerous and have raised serious concern among the medical scientists about the safety of the patients (Chattopadhyay, 2003). It is essential to establish internationally recognized guidelines for assessing their quality. The World Health Assembly in resolutions WHA31.33 (1978), WHA40.30 (1987) and WHA42.43 (1989) has emphasized the need to ensure the quality of medicinal plant product by using modern control techniques and applying suitable standards (WHO, 1998). The WHO has published guidelines in order to define basic criteria for evaluating the quality, safety, and efficacy of herbal medicines aimed at assisting national regulatory authorities, scientific organizations and manufacturers in this particular area (Akerele, 1993). However, the situation will change very quickly because the increase in the world market for medicinal herbs has attracted most of the largest pharmaceutical

companies, including some multinationals, and some of them have recently acquired small companies specialized in phytotherapeutic agents (Blumenthal, 1999^a; Blumenthal, 1999^b; Grunwald, 1995).

CONCLUSION

Among the functions of body healing of wound is a priority function. Early healing of wound is a field of attraction for research workers. Different topical applications are used to manage wound healing. Among the different topical applicants many herbal drugs are nowadays under trial to evaluate their wound healing efficacy. The use of herbal drugs is on a flow due to their safer nature and less frequent adverse effects as compared to conventional drugs. Different wound models ranging from partial to full thickness or incisional to excisional wounds in different animal models or even in cellular models have been used to evaluate wound healing efficacy of herbal drugs. Efficacy of herbal drugs has been evaluated using different parameters (Histopathological, bacteriological, hemato-biochemical). So far, herbal drugs in crude form as well as in different extract forms have proven to be efficient wound healing agents with less adverse effects and toxicities.

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Effect of Zinc Cyanide on the Behavior and Oxygen Consumption in Air Breathing Fish *Channa gachua*

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ABSTRACT

Gills are vital respiratory and osmoregulatory organs in fishes. Cyanide is a fast acting poison because it binds to key iron containing enzymes required for cells to use oxygen as a result the tissue are unable to take oxygen from the blood. In the present investigation an attempt has been made to study the impact of zinc cyanide on the behaviour and oxygen consumption in air breathing fish, *Channa gachua*. Short term acute toxicity test was performed by static renewal bio-assay test over a period of 96 hours, using different concentrations of zinc cyanide and LC50 value was found to be 343 ug / liter. It was observed that the normal respiratory activity (oxygen consumption) was significantly affected due to the depression in the metabolic rate at the end of the exposure periods i.e. 24, 48, 72 and 96 hours respectively. The fish *Channa gachua* in different toxic media shows passive drift, active upstream movement, loss of balance, hyper excitability, moving in spiral fashion with sudden jerky movement and rapid flapping of the opercular movement was recorded. The variation in the oxygen consumption in zinc cyanide treated fish is probably due to impaired oxidative metabolism and cyanide induced respiratory stress. Hence, dysfunction of behaviour and respiration can serve as index of toxicity in *Channa gachua*. The details will be dealt in this paper.

Keywords: Zinc cyanide, behaviour, oxygen consumption, *Channa gachua*

INTRODUCTION

The rapid industrialization of streams, lakes and rivers are receiving an increasing load of industrial wastes. Beside water pollution in many cases these waters kills the fish and other aquatic organisms. Fresh water are highly vulnerable to pollution since they act as immediate sinks for the consequences of human activity and always associated with the danger of accidental discharges. The ability to detect, identify and properly respond to natural chemical stimuli is an important component of the environmental physiology of fishes. Cyanide is fast acting poison because it binds to key iron containing enzymes required for cells to use oxygen and as results tissues are unable to take up oxygen from the blood. In the absence of first aid poisoning from gas inhalation ingestion or absorption through the skin can kill within minutes Gosselin *et al.*, (1976). Some of the cyanide is changed to thiocyanate, which is less harmful and leaves the body urine. Some can also combine with hydroxo cobalamine to form B12. A small amount of cyanide is converted in the body to carbon dioxide, which leaves the body within the first 24 hours after exposure (WHO, 1996). Cyanide is considered as a potent suicidal, homicidal, genocidal and chemical warfare agent. Cyanides may be released into the aquatic environment through waste effluents the organic chemical and gold mining and milling industries, as well as from industrial processes such as gas works, coke ovens gas scrubbing in steel plant, metal cleaning and electroplating. Cyanide in the aquatic environment may also be associated with non-point sources including runoff from application on land and water of salt containing cyanide compounds as anti-caking agent. Many cyanide containing compounds are highly toxic, but some are not. Nitriles [which do not release cyanide ions] and Hexa cyano ferrates [Ferrocyanide and Ferricyanide where the cyanide is already tightly bound to an iron] have low toxicities, while most other cyanides are deadly poisonous. These cyanides when dissolved in water they get dissociated and highly toxic free cyanide ion gets released, which get binds to the transition viz. copper and zinc forming the metal cyanide complex. Cyanide complex exists in

solution as an ionic cyano metallates and is highly stable. Zinc cyanide is an inorganic chemical compounds with the formula $\text{Zn}(\text{CN})_2$. It adopts a polymeric structure consisting of tetrahedral zinc centers linked by bridging cyanide ligands. It is employed as a catalyst for the cyanosilylation of aldehydes and ketones. It is also used to introduce the formyl groups in organic synthesis 2-Hydroxy-1Naphthaldehyde has been prepared from 2-Naphthol, zinc cyanide and anhydrous hydrogen chloride. Fish have become an indispensable model system for the evaluation of the extent of aquatic pollution. Fishes are used as biomarkers of not only acute toxic effect but also of the consequence of long term exposure to low concentration of pollutants Whitfield and Elliot (2002). Information on the acute toxic effects of metal cyanide on complexes in fishes is limited and its effects forms an important links in the aquatic food chain, are not known. The objective of the present study was to determine the acute toxicity of zinc cyanide in *Channa gachua* and its effect on behavioral and oxygen consumption. The reported result would be useful contribution in the ecotoxicity risk assessment studies of zinc cyanide on this fish species.

MATERIALS AND METHODS

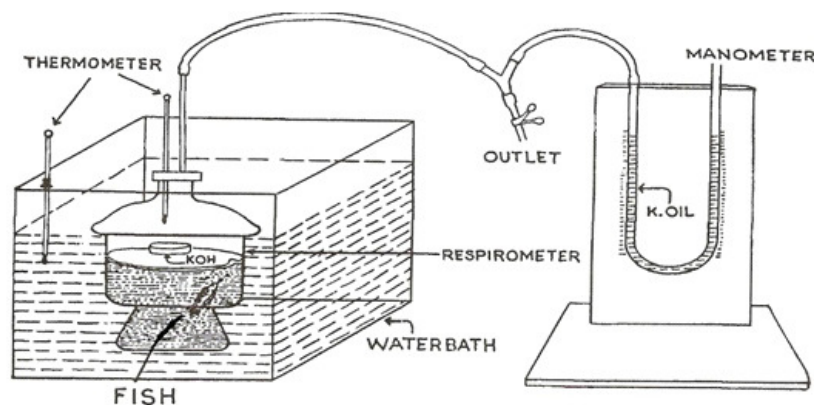


Fig. 1: Experimental set up for the measurements of dual mode of oxygen uptake in *Channa gachua*

Live specimens of *Channa gachua* were procured from local fish dealers at Hazaribag (Latitude 25° 59'N and Longitude 85° 22'E) and maintained in large glass aquaria size (90x60x60cm) 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 O₂ content = 6.5 mg O₂/litre; pH = 7.2) and 0.51 ML of air (Fig.1). 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 CO₂. 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 CO₂ 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 Winklers 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. 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 (Dejours, 1975). Mean values of VO₂ of 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 desired degree of concentrations was prepared by adopting the dilution techniques of APHA *et.al.*, (1971). The 96 hours bio assay tests were performed employing the technique of static bioassay tests (Doudoroff *et. al.*, 1951). Five fish were used for each set of

experiment and mean values of oxygen uptake of all the fish of each set of experiment were taken and compared. The experimental fishes including controls were divided into different groups each containing ten fishes. The animals of control group got the treatment of normal saline. The difference of significance, if any between the control and experimental groups of fish was calculated by students 't' test at the level of 5%.

RESULTS AND DISCUSSION

No mortality was observed in the control ones however, mortality increased with an increase in the concentrations and the exposure of duration. The concentration at which there was zero percent mortality was (335ug/L) in Table 1. The analysis of data from the present investigation evidenced that zinc cyanide is highly toxic and had profound impact on behaviour and respiration in *C. gachua*. Variation in the oxygen consumption in treated fish is probably due to impaired oxidative metabolism and cyanide induced respiratory stress, copious mucus secretion and bulging of gills were also observed. The drop in the oxygen consumption rate in *C. gachua* exposed to zinc cyanide can also be attributed to clogging of gills by mucous. These findings clearly suggest decreased respiratory surface dysfunction of behaviour and respiration can serve as index zinc cyanide toxicity.

The behaviour and condition of fishes in both the control and treated solution was noted in every 24 h to 96 h. The fishes showed marked changes in their behaviour when exposed to the test solution of different concentrations. In lower concentrations of zinc cyanide [335ug/L] the fishes showed rapid swimming than in control ones. Behavioural manifestations of acute toxicity like hyperactivity, loss of balance and rapid swimming increased surfacing activity was seen. For fish acclimatized to the 27°C temperature, the specific oxygen consumptions decreases with increase in zinc cyanide concentration. The oxygen consumption of fish exposed to zinc cyanide for 24, 48, 72

and 96 h of median lethal concentration was 0.3561, 0.3102, 0.2836 and 0.1837 (mg O₂/ L/h) respectively. Oxygen consumption increased in the initial 24 h of exposure to zinc cyanide concentrations. The oxygen uptake in *C. gachua* for 24, 48, 72 and 96 h was 0.3561, 0.3102, 0.2836 and 0.1837 (ml/kg/hr) the O₂ up take increased in the initial 24 h of exposure to zinc cyanide concentration. However, an average O₂ consumption in different time intervals of zinc cyanide exposure was significantly different from the control 0.4812 (mg/L). The decrease in O₂ consumption in *C. gachua* exposed to zinc cyanide indicates the onset of acute hypoxia under cyanide stress because of the drop in metabolic rate in fishes.

In the course of 96 h toxicity test in zinc cyanide to *C. gachua*, there was no mortality observed in control fish. The oxygen saturation of water neither drop below 60 percent in any concentration test, nor in the control groups. Presence of the substance tested [above 80 percent of the nominal concentration] was provided by the means of daily exchange of the testing bath. Where as in the present study the acute toxicity of zinc cyanide to *C. gachua* may be attributed the fact that cyanide induced changes in the physiological and survival of aquatic organisms under stress is complicated because such changes differ from compound to compound, species to species and from one experimental condition to another. The exact causes of death due to cyanide poisoning are multiple and depend mainly on time concentration combinations. However, there is no explanation on the exact mode of action of different metals causing the mortality in aquatic animals. Behavioral changes are most sensitive indication of potential toxic effects when studied. In the control groups the behavioural and swimming patterns of the fishes were normal and there was no mortality. The initial periods of exposure to zinc cyanide, the fishes stay in motion less and settled to the bottom. This can be attributed to the fact that, the sudden shock caused by the toxicant. The fishes began to swim naturally after an hour of exposure and the behavioural response

The shoaling behaviour was disrupted in the first day itself and they were spread out and appeared to be swimming independent of one another. The disturbance in the shoaling behaviour of the fish in the treated media indicates the loss of group hydrodynamic effect of fish. Zuyev and Bolyayen (1970) increased swimming activity and entails high expenditure of energy. Erratic swimming of the treated fish indicates the loss of equilibrium. Cyanide has profound effect on the central nervous system. This is strongly supported by the changes in the neurotransmitter levels in the corpus striatum and cerebellum. It is likely that the region of the brain which is associated with the maintenance of equilibrium might have been affected by the cyanide intoxication. Surfacing phenomena as observed in the fish treated with lethal concentration of zinc cyanide indicates hypoxic condition. Such surfacing might be to procure definite proportion of its oxygen requirement from the atmosphere. Loss of equilibrium follows erratic and darting swimming movements, which might be due to the inhibition of brain cytochrome C-oxidase activity, causing the brain damage to the region of the associated with the maintenance of equilibrium David *et al.*, (2007). In the study on the effects of eight selected organochlorine pesticides such as endosulphan, diazinone, phenyltrithion and methylparathion on eels, determined their 96 h LC₅₀ values and reported behavioral changes in the fish. They observed anxiety, disorders in swimming pattern, loss of balance, excessive mucus secretion and lightening in colour. Although the modes of function of these insecticides are markedly different than zinc cyanide, behavioural changes observed are similar to our study. Bardbury and Coast (1989) reported signs of fenvalerate poisoning in fish, which included loss of schooling behaviour, swimming near the water surface, hyperactivity, erratic swimming, seizures, loss of buoyancy, elevated cough rate,

increased gill mucus secretions, flaring of the gill arches, head shaking and restlessness before death. Such effects may be due to osmotic stress which affects the nervous system of the animal.

The present investigation demonstrated that despite the regulatory capability of the fish exposed to the toxicant, the oxygen consumption rate was indeed increased in the initial 24 h of exposure to lethal concentration of zinc [0.3561 mg/L] and after decrease in the oxygen consumption [0.1837mg/L] was noticed at the end of 96 h. Similar results have also been observed in different fish species for different chemical substances (Chinni *et. al.*, 2002 and Wu and Chen 2004). The decrease in the oxygen consumption is probably the result of alterations of energy metabolism. Some studies of the pathological effects caused by chronic exposure to chemical substances evidenced the gradual destruction of gills filaments, killing the fish by asphyxia (Zaccone *et. al.*, 1985). The oxygen consumption endpoint also provides an index for sublethal stress and for bio monitoring the potentially toxic effects of chemicals. Downing (1953) studied the effect of oxygen concentration on the toxicity of potassium cyanide to rainbow trout and revealed that, as the oxygen concentration increases the toxicity of potassium cyanide decreased. The decrease in the oxygen consumption in *C. gachua* exposed to zinc cyanide indicates the onset of acute hypoxia under stress. Further, the fact that the drop in metabolic rate of the fish as a protective measure to ensure a low intake of toxic substance that cannot be ruled out. Reduced oxygen consumption at higher concentrations of cyanide could also arise as result of respiratory inhibiting factors that come into play.

The primary site of action of cyanide is presumed to be the central nervous system. Cyanide acts through the inhibition of cytochrome C-oxidase in the respiratory electron transport chain of the mitochondria, impairing both oxidative phosphorylation Holland (1983) and Dreisenbach and Robertson (1987). Hence, a

number of other enzymatic processes are inhibited which exacerbate the toxicity and cyanides is also potent stimulators of events contribute to the acute toxic syndrome. 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. The observed increase in the OBF and TBF in the initial 24 h of zinc cyanide and decline after has been reported by Qaisur (2012). The initial increase in the OBF as a primary response to sudden stress was also reported by Rajasekaran *et. al* (2009). In the present investigation the initial increase may be attributed to the sudden shock caused by the toxicant. This elicits the potency and sensitivity in the fish *C. gachua* in the test chemical. The ecological importance is that the damage to non target species in the environment and such attribute of the organisms could be effectively used as toxicity, biosensor of chemical stress.

Table1: The mortality of *Channa gachua* in 96 hours at different concentration of zinc cyanide.

Conc(ug/l)	Log Conc.	No.fish exposed	Death	Mortality	Emp.probit
280	2.5250	10	1	10	3.72
285	2.5314	10	2	20	4.16
290	2.5327	10	3	30	4.48
291	2.5340	10	4	40	4.75
292	2.5352	10	5	50	5.00
293	2.5378	10	7	70	5.84
294	2.5403	10	8	80	5.93
295	2.5415	10	9	90	6.28
296	2.5440	10	10	100	8.09

Table 2: The opercular beat of *Channa gachua* in 96 hours at different zinc cyanide concentrations and time interval

Exposue time (Hr)	Control	96 h LC50 (343µg/L)
12	89.1±2.1	115±3.5
24	68.85±0.6	113±3.5
36	89.34±0.8	91.86±2.7
48	89.00±0.4	79.5±3.8
60	89.70±0.0	70.13±0.8
72	89.79±1.5	53.67±1.8
84	89.58±0.2	40±2.5
96	89.15±1.9	24.75±0.0

Table 3: The tail beat frequency of *Channa gachua* at different zinc cyanide conc. and time interval.

Exposue time (Hr)	Control	96 h LC50 (343µg/L)
12	8.2±0.8	16.4±0.5
24	8.2±0.1	15±3.5
36	8.7±0.6	14±2.7
48	8.6±0.2	11±3.8
60	8.3±0.5	8.5±2.1
72	8.5±0.3	5.1±2.8
84	8.1±0.9	4.3±0.6
96	8.0±0.2	1.9±3.5

Table 4: Oxygen consumption of *Channa gachua* at different conc. of zinc cyanide and time interval

Exposue time (Hr)					
Oxygen consumption	control	24	48	72	96
O ₂ /ml/kg/hr	0.4812	0.3561	0.3102	0.2836	0.1837
SD±	0.0001	0.0003	0.0003	0.0004	0.0003
% change	-----	-25.99	-35.53	-41.06	-61.82

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Composition of Essential Oils from the Leaf-margin of Lemon Balm

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ABSTRACT

The composite oil samples of leaf-margin of lemon balm (*Melissa officinalis* L.), on hydro-distillation provided a refreshing white viscous essential oil with characteristic lemon balm smell. The oil was found to be complex mixture of mono- and sesquiterpenes and 50 compounds comprising 98.95 % of the oil were characterized with the help of Gas Chromatography (GC), Gas Chromatography/Mass Spectrometry (GC/MS). Major compounds of the oil were characterized as caryophyllene oxide (12.5 %), β -pinene (11.2 %), γ -terpinene (10.3 %), and terpinene-4-ol (8.7%). This investigation performed on Lemon balm allowed the distinction of first sort of essential oils composition of, as far leaf-margin of Lemon balm is concerned.

Keywords: Lemon balm, leaf-margin, essential oils

INTRODUCTION

Lemon balm (*Melissa officinalis* L.) is an important aromatic plant, a perennial herb cultivated for lemon-scented leaves used as seasoning and in medicine. There are number of reports of literature on the essential oils of Lemon balm (Patora *et al.*, 2003). Mostly the volatile oil, its chemical profile; and its different pharmacological

activities such as antifungal, antibacterial and spasmolytic properties (Mimica-Dukic *et al.*, 2004; Larrondo *et al.*, 1995; Carnat *et al.*, 1998) are well documented. The alcoholic extracts of Lemon balm having antioxidant properties, normally because of high phenolic content such as rosmarinic acid (Mencherini *et al.*, 2007). Biosynthesis of proteins in cancer cells has been reported, by Lemon balm containing substances. Studies on the volatile oils of the balm is extensive but the action on other secondary metabolites is not so much in detail, but as far as leaf-margin there is as such no report of essential oils composition of Lemon balm.

MATERIAL AND METHODS

2.1. Plant source

The leaf of Lemon balm was collected from Bonera Field Station (Kashmir Valley) and identified by the taxonomist Dr Anzar Khuroo at Centre for Biodiversity and Taxonomy Biodiversity (CBT), University of Kashmir, Srinagar India.

2.2. Extraction and isolation

The essential oil was obtained by the hydro-distillation of fresh plant material in a Clevenger type apparatus for four hours. The sample afforded white viscous oil with characteristic lemon flavour (yield 0.04%). The oil was dried over anhydrous Na₂SO₄ and was placed at low temperature in refrigerator until analysis.

2.3. GC analysis

The composition of the oil was carried out by GC on a gas chromatograph Perkin Elmer-8500 with Flame Ionization Detector (FID), using a fused-silica column (30 m × 0.32 mm i.d.; 0.25 µm film thickness) coated with 5% diphenyl and 95% polysiloxane (BP-5). Oven temperature programmed from 60-220 °C. "Injector temperature, 240 °C"; "detector temperature, 270 °C". Carrier gas nitrogen at 8 psi, split ratio 1:80. Retention indices (RI) of the sample components and authentic compounds were determined on the basis of homologous *n*-alkanes hydrocarbons under the same

conditions.

2.4. GC/MS

GC/MS data obtained on Varian Mass Spectrometer using VF-5 column (60 m × 0.32 mm i.d.; 0.25 µm film thickness). Column temperature programmed 5 min. at 60 °C, then rising at 2 and 3 °C upto 240 °C. "Injector temperature, 240 °C"; "ion source temperature, 250 °C", "interface temperature, 270 °C; acquisition mass range 700-40 amu; ionization energy, 70 eV. Helium was used as carrier gas with a flow rate 0.5 ml/min. The identification of peaks was accomplished by comparison of the mass spectra with those reported in the NIST library (Adam, 1989). Identification of the oil components was also done by comparison of their linear RI with those from Mass Finder library.

RESULTS

The composite oil samples of Leaf-margin of Lemon balm (*Melissa officinalis* L.), on hydro-distillation provided a refreshing white viscous essential oil with characteristic lemon balm smell. The oil was found to be complex mixture of mono- and sesquiterpenes and 50 compounds comprising 98.95 % of the oil were characterized with the help of Gas Chromatography (GC), Gas Chromatography/Mass Spectrometry (GC/MS). Major compounds of the oil were characterized as caryophyllene oxide (12.5 %), β-pinene (11.2 %), γ-terpinene (10.3 %), and terpinene-4-ol (8.7%). The composition of the leaf-margin essential oils of Lemon balm (*Melissa officinalis* L.) is tabulated (Table 1).

Table 1. Composition of the leaf-margin essential oils of Lemon balm (*Melissa officinalis* L.)

Constituent	Retention Index	%age	Method of Identification
□-Thujene	926	3.4	MS, RI
□-Pinene	932	2.1	MS, RI
Camphene	936	0.03	MS, RI
Sabinene	942	2.3	MS, RI
Linalool	947	0.02	MS, RI
□-Pinene	984	11.2	MS, RI
Myrcene	1005	0.34	MS, RI
□-Phellandrene	1009	1.2	MS, RI
Citronellal	1079	4.8	MS, RI
Citranellol	1087	3.1	MS, RI
□-Terpinene	1092	1.0	MS, RI
p-Cymene	1098	0.01	MS, RI
Limonene	1128	0.5	MS, RI
Geraniol	1145	0.05	MS, RI
1,8-Cineole	1151	0.8	MS, RI
(Z)-□-Ocimene	1197	0.7	MS, RI
Neral	1213	0.008	MS, RI
Benzene acetaldehyde	1268	0.001	MS, RI
□-Terpinene	1392	10.3	MS, RI
cis-Sabinene hydrate	1415	1.2	MS, RI
Geranic acid	1431	1.3	MS, RI
Linalool	1447	0.04	MS, RI
trans-Sabinene	1461	0.02	MS, RI
□-Caryophyllene	1537	3.4	MS, RI
trans-Pinocarveol	1542	0.7	MS, RI
trans-Verbenol	1571	0.006	MS, RI
Geranylacetate	1627	2.03	MS, RI
Pinocarpone	1634	3.7	MS, RI
p-Mentha-1,5-dien-8-ol	1688	0.002	MS, RI
Terpinen-4-ol	1722	8.7	MS, RI
□-Humulene	1743	0.2	MS, RI
Myrtenal	1779	0.001	MS, RI
□-Cadinene	1856	0.01	MS, RI
Bornyl acetate	1872	1.3	MS, RI
Thymol	1897	2.5	MS, RI
Humulene oxide	1899	0.002	MS, RI
Carvacrol	1910	0.06	MS, RI
(E)-□-Damascenone	1912	0.05	MS, RI
□-Bourbonene	1918	1.2	MS, RI
(E)-Caryophyllene	1951	3.1	MS, RI
□-Humulene	1967	0.2	MS, RI
Germacrene D	1989	2.7	MS, RI
E-□-Ionone	2021	0.7	MS, RI
Bicyclogermacrene	2053	1.1	MS, RI
trans-□-Guaiene	2063	0.07	MS, RI
□-Bisabolene	2113	4.9	MS, RI
Caryophyllene oxide	2178	12.5	MS, RI
Caryophyllenol II	2185	1.2	MS, RI
Hexahydrofarnesyl	2191	0.2	MS, RI
Allyl acetate	2229	0.002	MS, RI
Total		98.952	

RI = Relative retention indices relative to C9–C23 *n*-alkanes on the BP-5 column. GC-MS identification based on comparison of mass spectra.

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Micronuclei Induction by Genotoxic Effects of Methyl-S-Demeton on Peripheral Blood Erythrocytes of *Cyprinus carpio*.

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ABSTRACT

Methyl-S- demeton, a pale yellow oily liquid with a penetrating smell, is a systemic and contact organophosphate insecticide used to control *Thysanoptera*, *Hymenoptera* and *Homoptera* in fruits, cereals, ornamentals and vegetables. To evaluate the Methyl-S-Demeton mediated genotoxicity on local fish of Kashmir, the micronucleus analysis was performed on peripheral erythrocytes of adult specimens of *Cyprinus carpio communis* and *Cyprinus carpio specularis* collected from the Dal Lake. Fishes were divided in two groups i.e. the control and the experimental groups. The experimental groups for each fish species were divided into three sub groups based on the selected doses of each insecticide. After treatment with each dose of insecticide, the frequency of micronuclei in all experimental groups was examined at the durations of 24, 48 and 72h. Three sub lethal concentrations of Methyl-S-Demeton, 2 ppm, 4 ppm and 6 ppm were used and it was observed that all these concentrations were able to induce micronucleus formation in erythrocytes of both fish species. Both dose and time dependent increase in micronucleus frequency was observed in treated fish species and a peak value detected at 72 h, higher concentration of Methyl-S-Demeton clearly showed a higher incidence of micronucleated peripheral erythrocytes.

Keywords: Methyl-S- Demeton, genotoxicity, *Cyprinus carpio communis*, *Cyprinus carpio specularis*, micronucleus test.

INTRODUCTION

Aquatic environment pollution is a serious and governing problem. In spite of legislation limiting the disposal of toxic chemicals, pollution of aquatic environments still occurs (Fleege *et al.*, 2003). Chemical contaminants with genotoxic and carcinogenic potential in the aquatic environments are a serious concern because they constitute a threat to aquatic as well as terrestrial life. These considerations have prompted interest in the development of techniques and bio indicators for monitoring genomic damage from hazardous contaminants in the aquatic environments (Dixon and Wilson, 2000). Among current genotoxicity test systems, the assessment of micronuclei is commonly used for evaluating structural and numerical chromosomal aberrations induced by clastogenic and aneugenic agents (Celic *et al.*, 2003). Micronucleus analysis was originally developed for mammals, but it has been successfully adapted for use in aquatic organisms especially fish (Cavas and Ergene-Gozyak, 2005). Because fish species constitute a vertebrate model, they have been widely used as a model organism in aquatic genotoxicity studies. Fish are considered sentinel organisms in a health assessment of aquatic environments (Dixon *et al.*, 2002). They have a great commercial and recreational value. Micronuclei have been induced in fish exposed to genotoxic substances under laboratory conditions and field conditions (Al-Sabti, 1991; Al-Sabti and Metcalfe, 1995; Russo *et al.*, 2004). Micronuclei (MN) are produced from fragments or entire chromosomes that lag in cell division because of a lacking or a damaged centromere or defect in cytokinesis. These small secondary structures of chromatin are surrounded by membranes, located in the cytoplasm and have no detectable link to cell nucleus (MacGregor, 1991; Seelbach *et al.*, 1993; Zoll-Moreux and Ferrier, 1999).

The run-off of insecticides from the agricultural fields to aquatic water bodies comprises a major part of aquatic pollution. This water pollution caused by such toxic chemicals is a matter of great human concern and warrants their testing for potential

genotoxic effect. During last few years, fishes have attracted much attention as laboratory animals for this type of study and attempts have been made to examine the peripheral erythrocytes in fish for the occurrence of micronuclei and using the information in a monitoring system for potential genotoxicity of an agent proposed (Hooftman and Raat, 1982; Manna *et al.*, 1985). However the protocol is yet to be standardised with different substances and in different piscine species.

In present study the incidence of micronucleus in the peripheral erythrocytes of *Cyprinus carpio communis* and *Cyprinus carpio specularis* treated with Methyl-S-demeton was analysed. The aim of the study was to assess the micronucleus test from the blood smear of fishes for detecting the possible genotoxic effect of Methyl-S-demeton which is formulated as an emulsifiable concentrate and used as a spray on cereals, fruits, ornamentals, vegetables and particularly used in agricultural fields of Kashmir against woolly aphids.

MATERIALS AND METHODS

Collection Site and Fish Collection

Adult specimens of *Cyprinus carpio communis* and *Cyprinus carpio specularis* were collected from the Dal Lake with help of local experienced fisherman and then these collected fish were transported to the laboratory in specially designed container having oxygen supply. After collection, fish specimens were acclimated for 45 days at 28°C prior to trials (Anitha *et al.*, 2000). Specimens were kept in propylene troughs with each 5-6 individuals/50 L of water. Water was kept O₂ saturated by aeration. The troughs were cleaned daily, and the water along with the insecticide was renewed to keep the concentration constant throughout the test period of 96 h. Water quality of the test solution was determined according to the standard procedures (APHA, 1998). The control fish were kept in experimental water (pH=7.3 ± 0.6; dissolved oxygen = 7.3 ± 0.4 ppm; free CO₂ = 5.8 ± 0.4; alkalinity = 106 ± 6.8 ppm) without adding these insecticides,

keeping all other conditions constant. Fish were fed daily with commercial feed at least one hour prior to the replacement of the water.

Selection and dosage of Methyl-S- demeton:

Commercial grade formulations of Methyl-S- demeton were used because only commercial preparations are used in agriculture. The commercial grade of Methyl-S- demeton was obtained from G.M.Shah pesticides (Srinagar), manufactured from Bhopal pesticides Ltd. (Bhopal India).

On the basis of literature data (LC_{50} values for each insecticide), three sub lethal concentrations of this insecticide was selected for the experiment as shown in table:

Organophosphate Insecticide	CAS		Concentration 1	Concentration 2	Concentration 3
Methyl-S- demeton	S-[(2 (ethylthioethyl) O, O dimethyl phosphorothioate.	-	2 ppm	4 ppm	6 ppm

Experimental Design:

In the experimental design, for the micronucleus assay fishes were divided in two groups i.e. the control and the experimental groups. The experimental groups for each fish species were divided into three sub groups based on the selected doses of insecticide. All groups had equal no. of fishes i.e., 5 fish per group in 50 L propylene troughs. After treatment with each dose of insecticide, the frequency of micronuclei in all experimental groups was examined at the durations of 24, 48 and 72h. Five fish specimens were used for each duration and at each concentration.

Analysis of micronucleus:

This micronucleus test was performed on peripheral blood according to the published protocols (Schmid, 1975; Hooftman and Raat, 1982; Al Sabti, 1986) with slight modifications as under:

Fishes were killed with a slight blow on the head region. Chemical treated and control

fish were cut in caudal region and smears of the peripheral blood were made on grease free clean glass slide. The simple hematoxylin and Eosin staining procedures of Pascoe and Gatehouse (1986) was employed with slight modification. The methanol fixed, air dried, smears were stained in filtered Mayer's Hematoxylin solution for approx. 5-10 minutes. The slide were then washed and rinsed in Scott's tap water substitute until the nuclear colour change (from red to blue) was completed as confirmed by microscopic examination. After completion of this blueing process, the slides were then washed in 30%, 50%, 70% and 90% alcohol. The slides were then stained in filtered Eosin solution for approximately 5 seconds or more. They were then again washed in 90% alcohol followed by washing in absolute alcohol. After dehydration, the slides were cleared in xylene and mounted using DPX. The slides were then examined using a simple light microscope (Olympus CX21) under low (600X) and high magnification (1000X).

Scoring of micronucleus

For each concentration and each duration five specimens were used and from each fish 6 slides were studied and 1200 cells (about 200 erythrocytes per slides) were scored under 600X magnification. Small non refractive, circular or ovoid chromatin bodies, displaying the same staining and focussing pattern as main nucleus, were scored. Particles with colour intensity higher than that of the main nuclei were not counted as micronuclei. Other nuclear abnormalities were also studied as classified by Carrasco *et al.*, (1990). Briefly, cells with two nuclei were considered as binuclei. Blebbed nuclei present a relatively small evagination of the nuclear membrane, which contains euchromatin. Nuclei with vacuoles and appreciable depth into a nucleus that does not contain nuclear material were recorded as notched nuclei.

The slides were carefully studied and various morphological peculiarities of the nuclear material were examined under light Trinocular microscope (Leica DMLS2) for accurate scoring of micronucleus.

Statistical analysis

Statistical analysis of data to verify the significant differences in the incidence of micronucleus between treated and control groups at 0.05 and 0.01 level of significance was performed using non-parametric criteria, Mann-Whitney U test to analyse the frequency of micronuclei. To ensure statistical accuracy, only cells with one micronucleus were considered, while rarely occurred two micronuclei and other nuclear abnormalities were eliminated from the counts. All the statistical calculations were done with the help of statistical software Minitab, V11.

RESULTS

The effect of Methyl-S- demeton on micronucleus induction was studied in *Cyprinus carpio specularis* and *Cyprinus carpio communis* following exposure to three sub-lethal different concentrations of (2 ppm, 4 ppm and 6 ppm) at 24h, 48h and 72 h. A dose response relationship was observed between the frequency of micronucleated erythrocytes and Methyl-S- demeton concentrations for *Cyprinus carpio specularis* and *Cyprinus carpio communis*. The peak frequency of micronucleated erythrocytes occurred at 72h exposure.

In *Cyprinus carpio specularis*, the percentage of single micronuclei (0.03 ± 0.01 of control) increased to 1.11 ± 0.37 from low to high concentrations by 24h and continued to increase by 1.92 ± 0.38 and 2.79 ± 0.56 in longer exposures (Table 1). Statistical analysis showed that all micronuclei frequencies significantly differ from controls ($P < 0.01$)

In *Cyprinus carpio communis* the incident of single micronucleus (0.03 ± 0.01) increased to 1.25 ± 0.356 from lower to higher concentration after 24hours and this value continued to increase by 2.18 ± 0.46 and 3.36 ± 0.37 after 48h and 72h respectively (Table 2). A statistically significant difference was observed among all treated groups in relation to control group ($P < 0.05$).

Table.1: Micronucleus frequency (%) in peripheral blood erythrocytes of *Cyprinus carpio specularis* exposed to different concentrations of methyl-S-demeton

Treatment	Concentration	MN frequencies (%)		
		24 h	48 h	72 h
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Control	-	0.03 $\pm$ 0.01	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01
Methyl-S- demeton	2ppm	0.04 $\pm$ 0.02	0.11 $\pm$ 0.02**	0.70 $\pm$ 0.61**
	4ppm	0.39 $\pm$ 0.05**	0.50 $\pm$ 0.16**	0.80 $\pm$ 0.11**
	6ppm	1.12 $\pm$ 0.37**	1.92 $\pm$ 0.38*	2.79 $\pm$ 0.5**

(Mann-Whitney U test)** P < 0.01

Table 2. Micronucleus frequency (%) in peripheral blood erythrocytes of *Cyprinus carpio communis* exposed to different concentrations of methyl-S-demeton

Treatment	Concentration	MN frequencies (%)		
		24 h	48 h	72 h
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Control	-	0.03 $\pm$ 0.01	0.04 $\pm$ 0.01	0.05 $\pm$ 0.01
Methyl-S- demeton	2 ppm	0.04 $\pm$ 0.01	0.138 $\pm$ 0.01*	1.95 $\pm$ 0.84*
	4 ppm	0.92 $\pm$ 0.01*	1.7 $\pm$ 0.42*	2.32 $\pm$ 0.70*
	6 ppm	1.25 $\pm$ 0.02*	2.18 $\pm$ 0.46*	3.36 $\pm$ 0.37*

(Mann-Whitney U test)* P < 0.05

DISCUSSION

Regulations in many countries are beginning to limit point source discharges of toxic chemicals into water resources, however, historical and current industrial and urban discharges are still responsible for high concentration of toxic substances in aquatic environments (Richards *et al.*, 2000). The potential genotoxic effects in aquatic organisms exposed to Phorate are poorly understood. Many contaminants present in aquatic environments not only endanger the survival and physiology of the organisms but also induce genetic alterations which may lead to mutations and cancer (Russo *et al.*, 2004). Future generations can be effected by reduced fitness and embryonic viability, along with genetic disorders (Kurelec, 1993). Insecticides may lead to changes in the blood biochemical parameters and haematological profile of fish which can be investigated as biomarker in pollution monitoring (Mushigeri & David, 2005; Banaee, *et al.*, 2008; Kavitha and Rao, 2009). Fish are often used as sentinel organism for

ecotoxicological studies because they play a number of roles in the trophic we accumulate toxic substances and respond to low concentration of mutagens (Cavas and Ergene-Gozukara, 2005). 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 (Lopez-Barea, 1996; Van Der Oost *et al.*, 2003). The frequencies of micronuclei in fish *Cyprinus carpio communis* and of *Cyprinus carpio specularis* from the world famous Dal Lake were observed in the study. Several studies have evaluated the genotoxic effects of methyl-S-demeton. Test results indicate that methyl-S-demeton is mutagenic. Mutagenic studies shows effects on sex chromosomes in fruit flies treated with 80 mg/kg of methyl-S-demeton. Microbes also mutated when exposed to 5 mg/kg of methyl-S-demeton (Smith, 1993). *In vitro*, methyl-S-demeton induced reverse gene mutations in *S. typhimurium* strains TA1530 and TA1535, but not in several other strains, such as TA1531, TA1532, TA1534, *hisC117*, and *hisG46* (Hanna and Dyer, 1975). Tested both with and without metabolic activation, methyl-S-demeton (purity: > 98%) was positive in *S. typhimurium* strains TA100 and TA1535 and negative in strains TA98 and TA100 (Herbold, 1980) while an overall positive result was reported for a 50.2% formulation using the same four strains (Herbold, 1979). Testing in several *E.coli* strains without metabolic activation resulted in positive results in strain WP2 *uvrA* only (not tested with metabolic activation) (Hanna and, Dyer, 1975). Methyl-S-demeton as a 53% formulation in xylene did not induce mutations in *S. cerevisiae* strains S138 and S211a when tested in the presence or absence of an S9 mix (Hoom, 1983). Methyl-S-Demeton induced recessive lethal mutations in *D. melanogaster* (Hanna and Dyer, 1975). A test for forward gene mutations in cultured mouse lymphoma L5178Y cells was positive at doses of 50-500 µg/mL in the presence and absence of metabolic activation (Cifone, 1984). In the present study the increased incidence of micronuclei was observed in peripheral erythrocytes of fish exposed to 2ppm, 4 ppm and 5 ppm of methyl-S-demeton.

In the present study the genotoxicity of Methyl-S-Demeton was tested for the

induction of micronucleus formation in peripheral erythrocytes of freshwater fish *Cyprinus carpio specularis* and *Cyprinus carpio communis*. The results revealed significant induction of micronuclei in the peripheral erythrocytes ($P < 0.01$ and $P < 0.05$) of *Cyprinus carpio specularis* and *Cyprinus carpio communis*. The appearance of interspecific differences observed could be due to the specificity of DNA repair, cell turnover time, physiological peculiarities, contaminate uptake or biotransformation in the fish species studied. A significant difference in the micronucleus incidence among treated and control groups were observed. The peak frequency of micronucleated erythrocytes was observed at 72 h after exposure. The length of the cell cycle critical to micronuclei formation depends upon the time needed to replicate DNA and perform nuclear division. In man and mice the duration of the cell cycle has been well documented.

The present study reveals micronucleus assay has a great potential for detecting clastogenic substances in aqueous media. Fish micronuclei assays represent a sensitive mean of measuring genotoxic activity in the laboratory. However, the accuracy of such assays in fish depends on improvements in certain parameters such as the number of cells scored, a better understanding of biochemical responses of fish to xenobiotics and knowledge of the erythrocyte cell cycle.

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***Trypanosoma mukasai* in the Fishes from Kashmir- a First Report**

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ABSTRACT

Schizothorax curvifrons Heckel, *Carassius carassius* Linnaeus, *Cyprinus carpio* Linnaeus and *Triplophysa marmorata* (Heckel, 1838) species of fishes were captured in Anchar Lake and river Jhelum of Kashmir Himalaya during December, 2008 to June, 2009 for parasitological investigation. During the analysis haemoflagellates from the genus *Trypanosoma* were recorded in blood smears. Trypanosomes were present in all the species except *C. carpio*.

Keywords: *Trypanosoma*, fish parasites, hematology, Kashmir waters

INTRODUCTION

Trypanosomes are haemoflagellates having a single free flagellum at the anterior end of the body. The first trypanosome was discovered from the blood of *Salmo trutta* by Valentin (1841). The parasite have been reported from fishes in different parts of the globe. e.g. *T. mukasai*, *T. froesi*, *T. satakei* and *T. britskii* from Brazil (Lopes *et al.*, 1991), *T. occidentalis* from Washington (Becker, 1967), *T. magdalenae* from Columbia, *T. acanthobramae* and *T. neinevana* were recorded from Iraq (Warsi and Fattohy, 1976).

From Indian, Qadri (1962) reported *T. batrachi* from *Clarias batrachus*; *T. gachuii* from

Ophiocephalus gachua (Misra *et al.*, 1973); *T. elongatus* from *Channa punctatus* (Raychaudhuri and Misra, 1973); *T. armeti* from *Mastacembelus armatus* (Mandal 1975); *T. trichogasteri* (Gupta and Jairajpuri, 1981), *T. colisi* (Gupta, 1986), *T. trichogasteri* var. *fasciatae* (Gupta *et al.*, 1998); *T. rohilkhandae* (Gupta and Saraswat 1991) and *T. sauli* (Gupta *et al.*, 2006) from *Channa punctatus*.

Most species of trypanosomes in fishes cause pathogenic diseases of considerable medical and economic importance. Symptoms of piscine trypanosomiasis range from mild anemia associated with low levels of parasitaemia to severe pathological changes due to heavy parasite burdens (Islam and Woo, 1991). Leukocytosis, hypoglycemia and hypocholesterolemia (Gupta and Jairajpuri, 1983) are frequent outcomes of trypanosomiasis.

Although a great number of parasitological investigations have been conducted on the fishes in Kashmir but most of the data pertain to ecto and endo-parasites mainly associated with the digestive system (Kaw 1950 and 1951; Fotedar 1958; Fotedar & Dhar 1973, 1974, 1977). Therefore, the piscine haemoparasites of fish infected with haemoparasites have not been investigated so far. Thus, the present study is aimed to report and identify the blood parasites of the genus *Trypanosoma* parasitizing freshwater fishes in Jhelum River and Anchar Lake, Kashmir.

MATERIALS AND METHODS

The study area was Anchar Lake and River Jhelum, Kashmir. Live fish belonging to four taxa namely, *Cyprinus carpio* Linnaeus, *Carassius carassius* Linnaeus, *Schizothorax curvifrons* Heckel and *Triplophysa marmorata* (Heckel, 1838) were collected from River Jhelum (34°04'17"N /74°49'08"E) and Anchar Lake (34°08'48" N /74°47'22" E) monthly between December, 2008 to June, 2009. On the field, the identification of the fish, and the collection of samples were carried out. Blood was collected from 210 live fishes from the caudal peduncle and heart as described by Lucky (1977). Part of the blood sample was used directly to make smears on grease- free slides for staining. For

determining haematology, samples were collected in glass vials containing EDTA as anticoagulant at an approximate concentration of 5mg/ml of blood (Blaxhall & Daisley, 1973).

Thin blood smears were made from the blood samples collected. The smears were air dried and fixed in absolute methanol. Slides were stained with Phosphate buffered Geimsa and examined under a microscope using a 100x oil immersion objective. Images were taken with the help of a Leica DM LS2 digital camera. Measurements were done according to Lom & Dykova (1992). Data were analyzed by using ANOVA and Student's T- Test.

RESULTS

In Giemsa-stained blood films, the body of the trypanosome stained deep blue, though its free flagellum, arising from the pointed anterior end of the body, was poorly stained. The kinetoplast was prominent, lying close to the blunt posterior end of the body (Fig. 1-3). The rounded nucleus stained pink with Giemsa and lay closer to the anterior end of the trypanosome than its posterior extremity. Morphometry and the number of fishes analyzed during are shown in Table 1.

No division stages were seen in the blood smears. Trypanosomes varied in size (Table 2, Figs. 1-3). Flagella were generally short and difficult to stain. Three Out of the four fish species investigated, i.e., *T. marmorata*, *S. curvifrons* and *C. carassius* were found to be infected with the trypanosome with an overall prevalence of 41.6%, 7.6% and 2.32% (Table 3), respectively. The trypanosomes (Figs. 1-3) from *T. marmorata*, *S. curvifrons* and *C. carassius* confirmed well to the description given for *T. mukasai*. The body length, body width and nuclear length of the trypanosomes described in this work are into the range of index quoted by Baker 1960. The nucleus lies forward of the midline (see Nuclear index values, Table.2), which supports its identity as *T. mukasai*.

Table 1. Morphometry and the number of fishes analyzed during present study

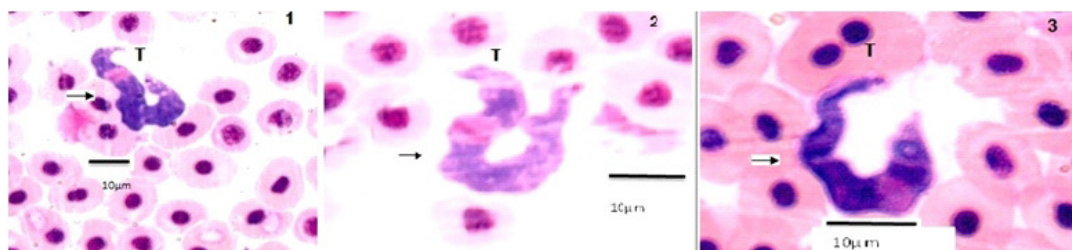
Fish	Number of sampled fishes	Length (centimeters)	Weight (grams)
<i>Cyprinus carpio</i>	55	21.73±2.15	190.83±53.99
<i>Carassius carassius</i>	55	14.39±2.17	58.99±31.85
<i>Schizothorax curvifrons</i>	60	23.05±3.16	128.05±58.11
<i>Triplophysa marmorata</i>	40	6.53±1.51	91.5±10.5

Table 2. Measurements of *Trypanosoma mukasai* (values as Mean + S.D) in μm from the fishes of Anchar lake and River Jhelum

Measurement	Length (μm)
Body length (BL)	36.63±4.35
Body width (BW)	4.64±0.88
Nuclear length (NL)	5.12±1.01
Nuclear width (NW)	3.86±0.5
Middle of nucleus to anterior extremity (AN)	14.42±2.62
Posterior extremity to middle of nucleus (PN)	21.71±3.1
Nuclear index (NI=PN/AN)	1.54±0.34
Kinetoplast to middle of nucleus (KN)	20.53±3.5
Kinetoplast Index (KI = PN/KN)	1.12±0.03
Total length (TL)	47.13± 3.1
Flagellar index (FI)	3.4

Table 3. Overall Prevalence of *Trypanosoma mukasai* in different fishes collected from Anchar Lake & River Jhelum

Parasite	Fish	Water body	Prevalence (%)
<i>T. mukasai</i>	<i>Triplophysa marmorata</i>	Anchar Lake	41.6
	<i>Carassius carassius</i>	Anchar Lake	2.32
	<i>Schizothorax curvifrons</i>	River Jhelum	7.6
	<i>Cyprinus carpio scapularis</i>	Anchar Lake	0
	<i>Cyprinus carpio communis</i>	Anchar Lake	0
	<i>Cyprinus carpio scapularis</i>	River Jhelum	0
	<i>Cyprinus carpio communis</i>	River Jhelum	0



Where, T stands for Trypanosome

Fig.1-3:- *Trypanosoma mukasai* in a Giemsa-stained blood film from different fishes in River Jhelum and Anchar Lake at 1000X

1) *Triplophysa marmorata*; 2) *Carassius carassius* ; 3) *Schizothorax curvifrons*

DISCUSSION

The higher infection prevalence of *T. mukasai* in *Triplophysa marmorata* when compared to other fishes in the same water bodies (Anchar Lake) seems to be attributable to the habitat preferences of these fish. *Triplophysa marmorata* may spend a lot of its time near or within vegetation, and this may make it highly exposed to infection and reinfection by leech bites. Leeches, once engorged with the blood of the host, detach and rest on a protected substrate (under a stone or in plant debris) in the water until the next meal (Paperna, 1996). This makes *T. marmorata* more prone to trypanosome infection than other fishes from the same habitat.

Baker (1960) found two morphological forms of *Trypanosoma mukasai* small (2244µm long) and large (4565µm) in the blood of fish. Baker (1960) commented that nuclear position, rather than flagellar length may be important in distinguishing the African freshwater fish trypanosomes. The nucleus lies forward of the mid-line consistently in the trypanosomes recorded in this study (see NI values, table 2), which support their identity as *T. mukasai*.

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Some Physicochemical Characteristics of Wular Lake, Kashmir

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ABSTRACT

The present study deals with the water quality of Wular Lake and the work was under taken from March 2012 to December 2012. The study reveals the changing trophic status of the lake being attributed to the sewage disposal and agricultural runoff by the catchment areas. The physico-chemical parameters of Wular Lake studied included the atmospheric temperature (recorded between 9°C to 27°C), hydrogen ion concentration (ranged between 7.4 to 8.1), dissolved oxygen (varied from 7.0 mg/l to 8.1 mg/l), free CO₂ (ranged from 10 mg/L to 14mg/L) ; total alkalinity (varies from 125mg/l to 160mg/l) ; ammonical nitrogen (from 80 µg/L to 102 µg/L); nitrate nitrogen (from 150 µg/L to 230 µg/L), orthophosphate (31 µg/L to 65 µg/L) and total phosphate (ranged from 183 µg/L to 243 µg/L). The high values of the physico-chemical parameters of water obtained in the present study indicate the eutrophic status of the lake.

Keywords: Water chemistry, lake, Kashmir.

INTRODUCTION

The Wular Lake is the largest freshwater lake of the Kashmir valley and has been designated as a Ramsar site in 1992. It is situated in northwest of Srinagar at distance of 35km. At the turn of the 19th century the area of lake was reported to be 217.8 km², which has got reduced to 86.71 km² by the start of 21st Century. The shrinkage in lake area was mainly due to continuous siltation brought about by various tributaries (Erin,

Madhumati, Ashtung) besides river Jhelum. The water body is very shallow, with the maximum depth of 5.8m. The lake is surrounded by high mountainous ranges on the northeastern and northwestern sides, which drain their runoff through various *nallas*, prominent being Erin and Madhumati. Geographically the lake is located at an elevation of 1580m (a.m.s.l) between the coordinates of 34°

15' - 34° 25' N Latitude and 74° 33' - 74° 44' E longitude. The Lake is an important habitat for the fish fauna of the region. The dominant fish species found in the Wular are: *Nemacheilus* sp., *Cyprinus carpio*, *Barbus conchoni*, *Gambusia affinis*, *Crossocheilus diplocheilus*, *Schizothorax curvifrons*, *S. esocinus*, and *S. niger*. Two species of *Triplophysa*, viz., *Triplophysa marmorata* and *T. kashmiriensis* have also been reported from this water body (Kullander *et al*, 1999).

Physico-Chemical parameters are highly important which directly or indirectly influence the distribution and abundance of species. Certain anthropogenic activities like discharge of domestic and agricultural wastes have increased the quantum of various chemicals that enter the receiving water, which considerably alter their physico-chemical characteristics. Phosphorous and nitrogen inputs from domestic wastes and fertilizers accelerate the processes of eutrophication (Rao and Valsaraj., 1984). Natural factors like dust, storm, runoff and weathering of minerals are slow processes in causing eutrophication (Kudari *et al.*, 2006). Eutrophication has become a widely recognized problem of water quality deterioration. The present study was therefore undertaken to monitor the water chemistry of the lake.

MATERIALS AND METHODS

The physico-chemical parameters of water were analyzed on seasonal from March 2012 to December 2012. The parameters like pH, temperature were monitored on spot while the parameters like free carbon dioxide, and alkalinity values were determined by APHA (1998). Nitrogen and phosphorus were calculated by Spectrophotometric method (APHA, 1998). For the collection of water samples

from the lake site Ningli (near the out let channel) with latitude $34^{\circ} 17' 15.8''$ N and longitude $74^{\circ} 30' 24.9''$ E was selected. Its depth ranges from 0.3 to 4.4 m.



Fig 1. A view of Wular Lake near study site In Ningli area

RESULTS AND DISCUSSION

The mean values for various physico-chemical parameters taken monthly at different sites during the entire study period are expressed seasonally and are summarized in Table 1.

Table 1: Physico-chemical parameters of Wular Lake

Parameters	Spring	Summer	Autumn
Water Temp. °C	9	23	17
pH	8.1	7.7	7.4
Dissolved Oxygen(mg/l)	7.6	7.0	8.1
Free Co ₂ (mg/l)	12	14	10
Alkalinity(mg/l)	160	125	132
Ammonical-Nitrogen(□g/l)	102	80	89
Nitrate-Nitrogen(□g/l)	150	200	230
Orthophosphorus(□g/l)	31	44	65
Total phosphorus (□g/l)	183	191	243

During the present study, water temperature fluctuated between a minimum of 9°C in the spring season to a maximum of 23°C in summer. Water temperature is the most important factor, which influences the chemical, bio-chemical and biological

characteristics of the water body. The variation in the water temperature of the present study is in broad agreement with the findings of Rao *et al.* (1982) for Nainital Lake (8 °C to 23 °C) and Billore and Vyas (1982) for Pichhola lake (0.6 to 26.3 °C).

The pH value was found to fluctuate from 7.4 to 8.1 at site Ningli in the season of autumn and spring respectively, indicating that the waters were neutral to alkaline. In case of Wular Lake, the high pH is due to the addition of hydroxyl, bicarbonate and carbonate anions¹⁰. This is in conformity with the observations of Zutshi and Khan (1977) and Zutshi and Vass (1978).

In any aquatic ecosystem, dissolved oxygen (DO) is of paramount importance because it is critical to the survival of most forms of aquatic life besides being the most reliable criterion in assessing the trophic status and the magnitude of eutrophication (Edmondson, 1966). Dissolved oxygen revealed a definite seasonal trend registering high values in autumn (8.1mg/l) and low in summer (7.0 mg/l). Low values of DO imply higher trophic status (Naz and Turkmen, 2005). Similar types of results were observed in present study as dissolved oxygen decreased with increase in temperature. It is regulated primarily by free diffusion of oxygen air to water, production through photosynthesis, consumption by biota, dissolved oxygen affects the solubility and availability of many nutrients and therefore productivity of aquatic ecosystem (Wetzel, 1983). Highest value may be due to cooling (Hunnam, 1979). Reduction in the value observed with increase in temperature, which attributed to high microbial activity. Low content of dissolved oxygen though a sign of organic pollution is due to inorganic reductants like hydrogen sulphide, ammonia, nitrates and ferrous ions.

During the present study concentrations of free CO₂ were noticed which ranged

between 10 mg/l to 14 mg/l respectively in the autumn and summer season. The high value of the free carbon dioxide content is an indication of high degree of pollution, a fact also supported by Todda (1970) and Coole (1979) which related high value of free carbon dioxide content to high degree of pollution.

Alkalinity varies from 125 mg/l to 160 mg/l. It was maximum in the season of spring and minimum in the season summer. Alkalinity of water is a measure of weak acid present in it and of the cat ions balanced against them. Venkateswarlu (1969) attributed that there is an indication to suggest that alkalinity concentration is affected directly by rainfall.

Phosphorous, is generally recognized as one of the key nutrients in the productivity of freshwaters as it is essential element determining fertility of lakes. The concentration of orthophosphate phosphorus (OPP) during the study period ranged from a minimum of 31 µg/l in spring season to a maximum of 65 µg/l in autumn season. The low orthophosphate-phosphorous content in waters is due to the formation of an insoluble calcium-phosphate complex. Such a phenomenon functions as scavenger of some inorganic nutrients and also acts as a removal agent of dissolved organic matter by absorption (Otsuki and Wetzel, 1974).

The fluctuations regarding the total phosphate phosphorous (TPP) were irregular. In general, lower concentrations were observed during spring season (183µg/l) and higher concentrations during autumn season (243µg/l). The total phosphorus and the orthophosphate content in the Wular Lake fluctuated greatly during the course of the year. However, the average concentration of both total phosphorus and orthophosphate phosphorus revealed the water body belonging to the hypertrophic category of Wetzel (1983). This is substantiated by the fact that almost the whole water body is infested by the macrophytes, which is possible only when this important nutrient is available in ample quantities. Bandela *et al.* (1999) observed an increase in phosphate concentration in those water bodies that received domestic waste.

The values of Ammonical nitrogen ranged from 80 µg/l in summer season to 102 µg/l during spring season. Such fluctuations in the values of ammonical nitrogen may be due to decomposition of organic matter and bird droppings into the lake as it is visited by many aquatic birds (Zuber, 2007). Prasad (1990) pointed out that ammonical nitrogen increases during rainy seasons.

Nitrate nitrogen (NO₃-N) was higher in the autumn season (with maximum value of 230 µg/l) while the lower values were recorded in the spring season (with minimum value of 150 µg/l). Ganapati (1960) pointed out that the concentration of nitrate-nitrogen (>150µg/l) is an indicative of eutrophication and as such the Wular lake falls in eutrophic category.

In conclusions it is inferred that the effluents and agricultural run off released and other anthropogenic disturbances are responsible for changing tropic status of the lake. Therefore preventive measures are required particularly at state level to safe guard this indispensable aquatic ecosystem.

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Impact of Nematode Parasites on Hematological Parameters of Goats

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ABSTRACT

The aim of present study was to compare the prevalence of various nematode infections and their impact on hematological parameters of goats in South Kashmir. Hematological estimations and faecal egg counts of 180 local goats sampled from different districts of South Kashmir during December 2011-October 2012 was carried out. Out of these, 110(61.11%) were found infected, which in decreasing order are the species of *Haemonchus*, *Ostertagia*, *Nematodirus*, *Trichuris*, *Bunostomum*, *Trichostrongylus*. Infection rate in younger animals was higher than adults and was maximum in summer and lowest in winter. Out of 180 samples taken, 42 were taken for hematological analysis which showed prevalence of mixed infection. These were found highly infected, having mixed infection. The hematological study of nematode infected goats showed a significant decrease in red blood cell (RBC) count, hemoglobin (Hb) concentration, percentage of packed cell volume (PCV%). Moreover, white blood cell (WBC), lymphocytes and neutrophil counts were significantly increased in infected goats.

Keywords: Hematological parameters, prevalence, nematodes, goat.

INTRODUCTION

Goat also known as “poor man's cow” is generally reared for meat, milk and wool purposes. About 20 specific goat breeds are known to exist in India (Acharya, 1988). Kashmir is primarily an agricultural state and animal rearing is one of the major sources of earning of farming community and goat farming is an important source of livelihood for small and marginal farmers and landless village dwelling community, as it plays an important role in providing food, fibre, manure etc. Goats play an important role in the economy of J&K state. These are generally reared by poor and backward classes like nomads, Gujjars, Bakerwals etc., who take them to various grasslands, pastures for purpose of grazing. During this process the goats are infected by a large number of parasites.

Gastro-intestinal parasitism represents a severe health problem in small ruminant production system, especially in sheep and goats and its consequences can be extensive ranging from reduced productivity to mortality (Skykes, 1994). It may also cause body composition changes and rendering the affected animals more susceptible to concurrent infections (Dominguez-Torano *et al.*, 2000). Helminthiasis is one of the most important animal diseases worldwide, inflicting heavy production losses in grazing animals. The disease is especially prevalent in developing countries (Dhar *et al.*, 1982) mainly as a result of poor management practices and inadequate control measures. Gastro-intestinal nematodiasis is a major threat and a primary constraint to sheep productivity, it endangers animal welfare worldwide (Tariq *et al.*, 2008). The prevalence of GIN in tropical and sub-tropical areas has adversely affected the production potential of sheep and goats, leading to countless deaths and insidious economic losses in livestock sector (Al-Quaisy *et al.*, 1987). They cause significant economic losses worldwide due to their feeding behaviour being haematophagous like *Haemonchus contortus* and *Ostertagia ostertagi* suck 0.05ml blood/worm/day (Soulsby, 1986).

Blood is an important and reliable medium for assessing the health status of individual animals (Oduye, 1976). Hence the present work was aimed at to investigate the prevalence of nematode parasites and to correlate them with the Hematological studies of goats of south Kashmir.

MATERIAL AND METHODS

Fresh faecal samples were collected from rectum of goats. Samples were collected in suitable plastic bags and carefully labeled with animal identification, age, sex, and date of collection. Samples were preserved at 4°C until analysed. Will's floatation technique was used for qualitative analysis of the faecal samples and Mc Master egg counting technique for quantitative analysis (Soulsby, 1986).

Blood samples were collected from the external jugular vein of goats in EDTA containing vials. Total erythrocyte, leukocyte and platelet counts were made using an improvised Neubauer's haemocytometer. The percentages of neutrophils, eosinophils, basophils, monocytes and lymphocytes were determined from differential counts of leukocytes on fixed and stained whole blood films and these data were converted to total cell counts for each cell type. Packed cell volume (PCV) was determined using the microhematocrit method, and hemoglobin (Hb) concentration was measured by the cyanmethemoglobin method. Statistical analysis was carried out by SPSS 16.5 (2011) software. Results are expressed as means $\pm$ SEM (standard error of the mean). Prevalence was calculated by the percentage of infected animals.

RESULTS

Prevalence analysis:

Out of 180 faecal samples of goats examined, 110(61.11%) were found infected. The most commonly nematode parasites found were *Haemonchus* sp. (62%), *Ostertagia* sp. (44%), *Nematodirus* sp. (40%), *Trichostrongylus* sp. (38%), *Bunostomum* sp. (35%), *Trichostrongylus* sp. (30%). Age wise prevalence of nematode parasites in goats is presented in table1. The highest infection (83.33%) was found in age-group <1 year,

least prevalence (31.57%) was found in older age groups. Seasonal prevalence of nematode infection in goats was found maximum during summer (80.95%) and lowest (27.77%) in winter (Table 2). The prevalence rate increases gradually from spring to summer 62.06% - 80.95% respectively.

Table 1. Age wise distribution of Nematode infection in goats

Age group(Years)	No. Examined	Positive (%)
< 1	48	40(83.33)
1-2	52	36(69.23)
2-3	42	22(52.38)
>3	38	12(31.57)
Total	180	110(61.11)

Table 2. Seasonal Prevalence of Nematode infection in goats

Seasons	No.Examined	Positive (%)
Winter	36	10(27.77)
Spring	58	36(62.06)
Summer	42	34(80.95)
Autumn	44	30(68.18)

Hematological analysis

42 blood samples were taken for hematological analysis. Results showed reduction in RBC counts, Hb concentration and PCV in infected animals which can be attributed to the loss of blood by sucking activity of parasites mainly because of *Haemonchus* sp. and *Ostertagia* sp. Similar results were determined by (Soulsby, 1986). Moreover the present study showed the mean total WBCs, lymphocytes, neutrophil and eosinophil counts were significantly higher in infected animals (Table 3). Eguale and

Abie (2003) detected the similar results. Hemoglobin concentration decreased markedly in infected goats i.e

11.11 g/dl - 8.44 g/dl. Lymphocyte number significantly increased in infected goats from 59.11% - 66.27%. Infected goats were found anemic.

Table 3. Hematological parameters of uninfected and infected goats

Components	Uninfected values (Mean±SEM)	Infected values (Mean±SEM)
Hb (g/dl)	11.11±0.30	8.44±0.41
PCV (%)	29.22±1.14	27.05±1.12
TEC (10 ⁶ /cumm)	11.66±0.63	9.11±0.42
TLC(10 ³ /cumm)	8.11±0.52	9.77±0.54
Lymphocytes (%)	59.11±2.57	66.27±2.43
Neutrophils (%)	33.94±1.91	34.88±1.85
Eosinophils (%)	4.16±0.53	5.05±0.62

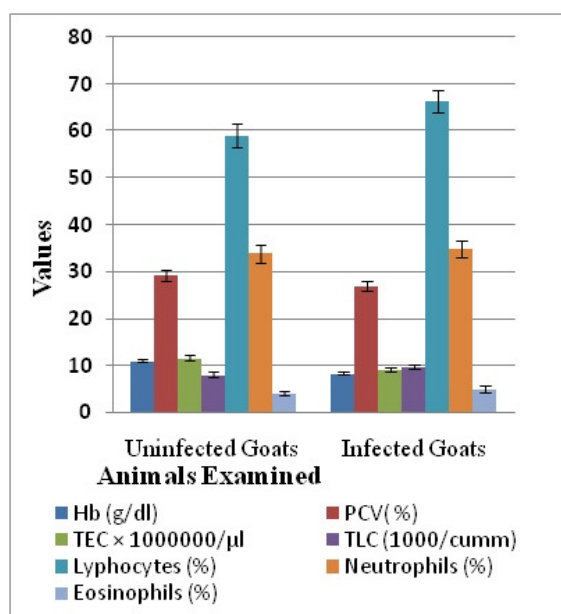


Fig1. Hematological parameters of infected and uninfected Goats

DISCUSSION

The occurrence of nematodiasis in an area is influenced by a multifactorial system, involving mainly the particular host, parasite and their environment. *Haemonchus contortus* and *Ostertagia Ostertagi* being common blood feeders that cause anaemia and reduced productivity and can lead to death in heavily infected animals. It has been estimated that each worm sucks about 0.05 ml of blood per day by ingestion or seepage from lesions (Urquhart *et al.*, 2000).

The present study indicates that the nematodiasis is a frequent process and one of the major parasitic problems of the small ruminants. These findings are consistent with those of Tariq *et al.* (2008); Lone *et al.* (2011); Dhar *et al.* (1982); Makhdoomi *et al.* (1995); Agyei, (1991); Nginyi *et al.* (2001); Shahadat *et al.* (2003); Lateef *et al.* (2005); Nwosu *et al.* (2007).

During present study nematode infection was known to cause significant changes in hematological parameters like Hb%, PCV and RBC count and result in anemia in infected animals. Similar results has been determined by Siham *et al.* (1997); Sharma *et al.* (2000); Taleb *et al.* (2007); Abel-Nabi *et al.* (2002). Thus the nematode infection causes the significant impact on the blood physiology of goats. The present study will be helpful in finding the physiological and health status of goats. It will also be useful in early diagnosis of nematode infection in goats and a treatment schedule could be designed to avoid more infection and animal losses on the farm level and in turn economical losses. Thus there is an urgent need to carry out research on applied aspects leading towards control strategies of parasites. Regular deworming and improvement of husbandry practices is suggested.

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Major Elemental Chemistry of the Part of River Jhelum, Jammu and Kashmir (India): Weathering Processes and Irrigation Quality Assessment

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ABSTRACT

This paper presents a study on the part of River Jhelum, which is one of the longest rivers in Kashmir Valley, India. Eleven (11) water samples were analyzed, to assess Weathering process and irrigation quality of the river. The river water was found to be controlled by chemical weathering of the rock forming minerals. Scatter diagrams suggested the dominance of carbonate and silicate weathering. Two specific types of water were identified with the help of Durov and piper diagrams that are referred to as CaHCO_3 , MgHCO_3 types. The langlier-diagram confirms the chemistry of meteoric water, i.e; Ca-Mg-HCO_3 . The calculated values of SAR, RSC and sodium percentage indicated that the river water is excellent for irrigation use.

Keywords: Jhelum, River, weathering, scatter, Durov, piper, meteoric water, irrigation

INTRODUCTION

India has a large river network which has been nurturing its vast fertile lands. The rapid urbanization, industrialization, intensive agriculture and growing demand for energy have adversely affected the physiochemical parameters of surface water (Jain *et al.* 2007). The groundwater level is declining continuously and thus increasing the

dependency of people on surface water resources. Therefore analysis of river water quality is very essential for sustainable use of river water resources. The river water quality in a region is largely determined by the natural processes viz. precipitation rate, weathering processes, soil erosion etc. as well as anthropogenic processes viz. urban, industrial, agricultural activities and increasing exploitation of water resources (Carpenter *et al.* 1998). The municipal and industrial wastewater discharge (point as well as non-point sources of pollution) constitutes the constant polluting source of river water quality. The surface run-off during the rainy season also affects river water quality (Kazi *et al.* 2009). The hydrogeochemistry of river water is controlled by a series of factors such as climate, vegetation, topography and geology of the catchment area (Alaez *et al.* 1988). The river water ecosystem is affected by fluctuations in physical and chemical characteristic of river (Guissani *et al.* 2008).

Major chemical composition of river water e.g. (Ca, Mg, Na, K, HCO_3 , SO_4 , Cl) can reveal the nature of weathering patterns and anthropogenic processes (Gibbs, 1970). Quantifying the major-ion composition of river water also has broad implications, e.g. water quality type, hydrogeology characteristics, weathering processes (Brennan and Lowenstein, 2002; Cruz and Amaral, 2004). Many previous studies have revealed the major-ion chemistry of the world's rivers, e.g. the Amazon (Gibbs, 1972; Stallard and Edmond, 1983, 1987), the Orinoco (Nemeth *et al.*, 1982), the Yangtze River (Chen *et al.*, 2002), the Yellow River (Zhang *et al.*, 1995; Chen *et al.*, 2005) and the Ganges Brahmaputra (Sarin *et al.*, 1989) amongst others.

In the present work, a detailed hydrogeochemical study of river Jhelum has been carried out to determine the major ion chemistry and to understand the weathering and geochemical processes controlling the water composition and suitability of water for irrigation purposes.

Study area

After the origin of Jhelum River from Pir Panjal range of mountains it flows through Kashmir valley in north westerly direction till it falls into the Wular Lake in Baramulla District. After its re-emergence from the Wular Lake in Sopore (fig.1), it takes a Southwesterly direction and continues its journey through Uri before entering the Pakistan occupied Kashmir.

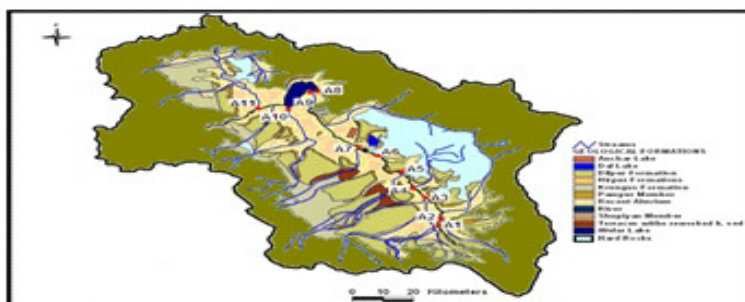


Fig.1. Map of River Jhelum and sampling locations

Sampling and Analysis

Water samples were collected from River Jhelum during summer 2010. The samples were filtered using 0.45 μm nylon membrane Millipore filters. The standard methods were adapted to analyses (APHA, AWWA and WEF 2001). Temperature, pH, conductivity (EC) and alkalinity were measured at site. The major ion analysis was carried out at the Geochemistry Lab of Department of Geology and Geophysics, University of Kashmir, Srinagar. Alkalinity was measured by HCl titration; Ca^{2+} and Mg^{2+} by EDTA titration; Cl^- by AgNO_3 titration; SO_4^{2-} by spectrophotometry; Na^+ and K^+ by flame emission photometry. *In most of the water samples, the total cation charge ($\text{TZ}^+ = \text{Ca}^{2+} + \text{Mg}^{2+} + \text{Na}^+ + \text{K}^+$ in meq/l) balances that of the total anions ($\text{TZ}^- = \text{HCO}_3^- + \text{Cl}^- + \text{SO}_4^{2-}$ in meq/l) within analytical uncertainties and the normalized inorganic charge balance ($\text{NICB} = (\text{TZ}^+ - \text{TZ}^-)/\text{TZ}^+ \times 100\%$) is within $\pm 5\%$.*

RESULTS AND DISCUSSIONS

Physico-chemical characteristics of river Jhelum

Summary of physico-chemical analysis of water samples from river Jhelum is presented in Table.1. The river waters are fresh, colorless, and odorless with lower *temperature* (T range: 17.8°C-19.3°C, mean: 18.5, standard deviation: 0.371). As expected, the river waters are alkaline (pH range: 7.5-7.8, mean: 7.6, standard deviation: 0.1). Medium electrical conductivity (EC range: 195.3-279.6 μ S/cm, mean: 254, standard deviation: 18.3). Medium total dissolved solids (TDS range: 125-179 mg/l, mean: 162.6, standard deviation: 11.7). Calcium concentration ranged from (24-35 mg/l) Mean: 30.7, Standard deviation; 3.2. Magnesium concentration ranged from (4.4-14.5 mg/l) Mean: 8.7, Standard deviation: 2.9; Bicarbonate concentration ranged from (112-178 mg/l), Mean: 140.2, Standard deviation; 15.5); chloride concentration ranged from (3.6-8 mg/l) Mean: 5.4, Standard deviation: 1.2 and sulphate concentration ranged from (5.4-9.8 mg/l) Mean: 8.0, Standard deviation: 1.6. Among the cations, Mg^{2+} and Ca^{2+} were most abundant and the general order of major cations was $Ca^{2+} > Mg^{2+} > Na^{+} > K^{+}$. Among the anions, HCO_3^{-} was most abundant and the general order of major anions was $HCO_3^{-} > SO_4^{2-} > Cl^{-}$.

Table.1. Physio-Chemical analysis of River Jhelum Waters

Location	Site ID	Temp. C°	pH	E.C μ S/cm	T.D.S mg/l	Ca ²⁺ mg/l	Mg ²⁺ mg/l	Na ⁺ mg/l	K ⁺ mg/l	Cl ⁻ mg/l	HCO ₃ ⁻ mg/l	SO ₄ ²⁻ mg/l
(Kurshepur) Khanabal	A1	17.8	7.5	195.3	125	35	13.1	10.4	0.36	8	178	6.4
(Gur) Khanabal	A2	17.8	7.5	218.7	140	24	14.3	8.6	0.29	6	145	9.8
Sangam	A3	18.4	7.7	223.4	143	32	10	9.4	0.24	7	154	5.6
Kakapora	A4	18.4	7.6	231.2	148	28	4.4	8.4	0.29	4.6	112	7.6
Awantipora	A5	18.8	7.8	240.6	154	24	9.2	8	0.33	5	126	5.4
Srinagar (Amirakadil)	A6	19.3	7.5	248.4	159	34	4.9	6.6	0.25	3.6	127	9.8
Shadipora	A7	18.9	7.7	257.8	165	34	6.8	7.4	0.3	5	140	7
Bunyar	A8	18.4	7.7	264	169	28	14.5	9.4	0.3	4	165	9
Gulamyar	A9	18.4	7.7	265.6	170	31	10.7	9.1	0.44	6	151	9
Sopore (A)	A10	18.1	7.6	275	176	34	9.2	8.6	0.43	7	149	9.4
Sopore (B)	A11	18.1	7.5	279.6	179	31	8.7	8.4	0.46	6.3	138	9.4

Major ion composition

The major ion chemistry of groundwater is a powerful tool for determining solute sources and for describing water evolution as a result of water-rock interaction leading to the dissolution of carbonate minerals, and silicate weathering and ion exchange processes (Herczeg *et al* 1991; Hiscock 1993; Kimblin 1995; Elliot *et al* 1999; Edmunds and Smedley 2000; Jeelani and Shah 2006). Gibbs (1970) gave a relation for determining the major mechanism controlling water chemistry, which suggested that the major mechanism controlling the water chemistry of river Jhelum is the chemical weathering of the rock forming minerals (TDS:125179 mg/l and weight ratio of Na/(Na+Ca):0.1620.263) (fig.2)

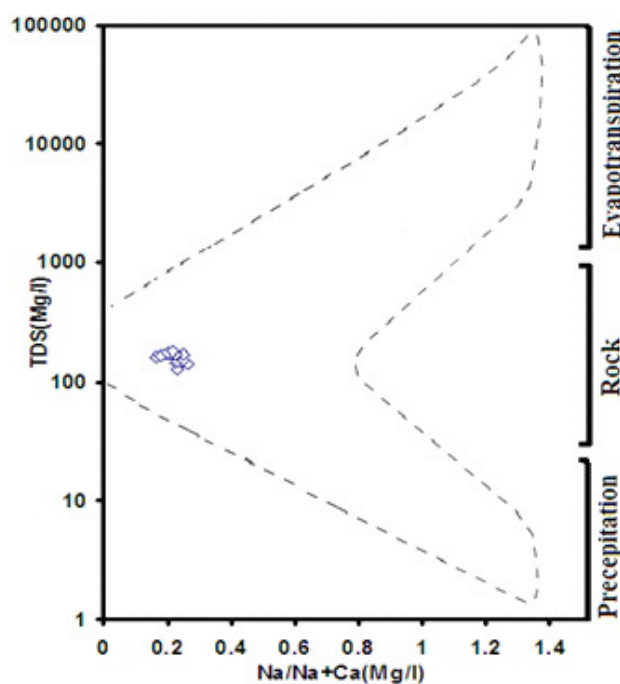


Figure. 2. Gibbs diagram

Evolution of water and relationship between rock types and water composition can be evaluated by the Piper trilinear diagram (Piper, 1944), which is very useful in determining chemical relationships in water in more definite terms than possible with other plotting methods (Walton, 1970). The piper diagram is an ingenious construction, which consists of two triangular diagrams at the lower left and lower right, describing the relative composition of cations and anions and an intervening diamond-shaped diagram that combines the composition of cations and anions. River water samples were plotted on piper trilinear diagram (fig.3.), which reveals that General chemical water type identified was Ca Mg HCO₃ Type and specific water types were Ca-HCO₃ and Mg-HCO₃.

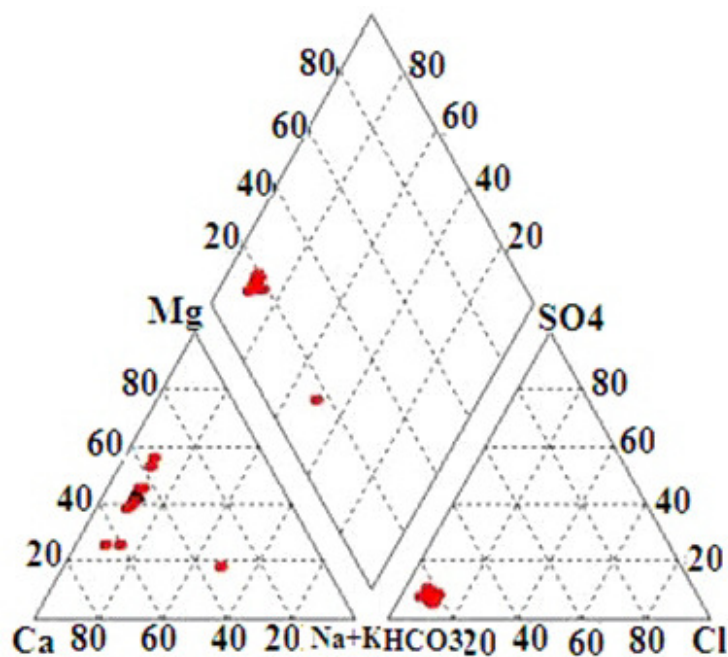


Fig. 3. Piper trilinear diagram showing broad water types

The Durov diagram (Durov 1948) plots the major ions as percentages of milliequivalents in two base triangles. The main purpose of the Durov diagram (Durov 1948) is to show clustering of data points to indicate samples that have similar compositions. Chemical facies that determine the water type are calculated by first converting the concentration (meq/l) of the major cations (Na^+ , K^+ , Ca^{2+} , Mg^{2+}) and anions (Cl , SO_4^{2-} , HCO_3^-) to percentages (Guler *et al.* 2002). The Durov plot (fig.4) indicates dominance of the major ions Ca^{2+} , Mg^{2+} , HCO_3^- while other ions, such as SO_4^{2-} , Na^+ , K^+ and Cl , are comparatively less represented, indicating weathering inputs in the water system (Cetindag and Okan 2003). All the samples chop in Ca-Mg- HCO_3 facie, which can be subdivided into Ca HCO_3 and Mg- HCO_3 facies. Ca HCO_3 facie indicates the dominance of alkalies and weak acids. Mg- HCO_3 facie again indicate that strong acid i.e Cl and SO_4 does not exceed the weak acids (HCO_3^-).

Geochemical processes controlling water composition

Binary plots were plotted to study the weathering regimes and dominance of major ions $\text{Ca}^{2+} + \text{Mg}^{2+}$ is plotted against HCO_3^- (fig.5a) all the points fall below 1:1 equaline suggesting some contribution from silicates or/and sulphates. In the plot of $\text{Ca}^{2+} + \text{Mg}^{2+}$ Vs $\text{Na}^+ + \text{K}^+$ (fig.5b) all the points fall below 1:1 equaline indicating carbonate lithology as the dominant source of major ions. (Das; 2001 C Own Wlr ppr). In the plots of $\text{Ca}^{2+} + \text{Mg}^{2+}$ Vs $\text{HCO}_3^- + \text{SO}_4^{2-}$ (fig.5c) all the points fall near/or above 1:1 trend line indicating carbonate lithology as the main contributor of major ions with some contribution from silicate lithology (Jeelani and Shah; 2006, Sarin *et. al* 1989) as Ca^{2+} is derived mainly from carbonates with some inputs from silicates.

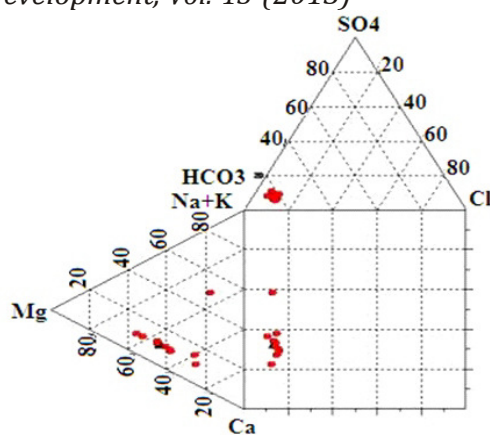


Fig. 4. Durov diagram

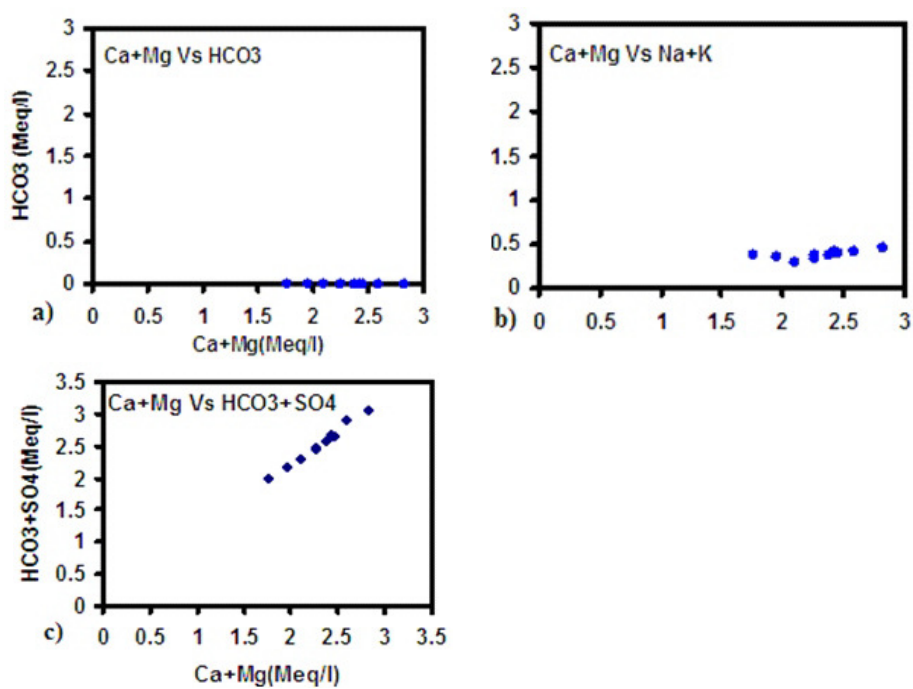


Fig. 5. Scatter diagrams between (a) Ca+Mg vs HCO₃; (b) Ca+Mg vs Na+K; (c) Ca+Mg vs HCO₃+SO₄ showing possible liganding of the major ions.

The langlier-diagram helps arrive at closer classification of waters. Analyzed water samples from river Jhelum (fig.6), confirm the chemistry of meteoric water, i.e; Ca-Mg-HCO₃ type, however a sample showed deviation from core end. The alteration of meteoric water to different chemical composition waters is due to the maximum waterrock interaction (Umar *et al*, 2006).

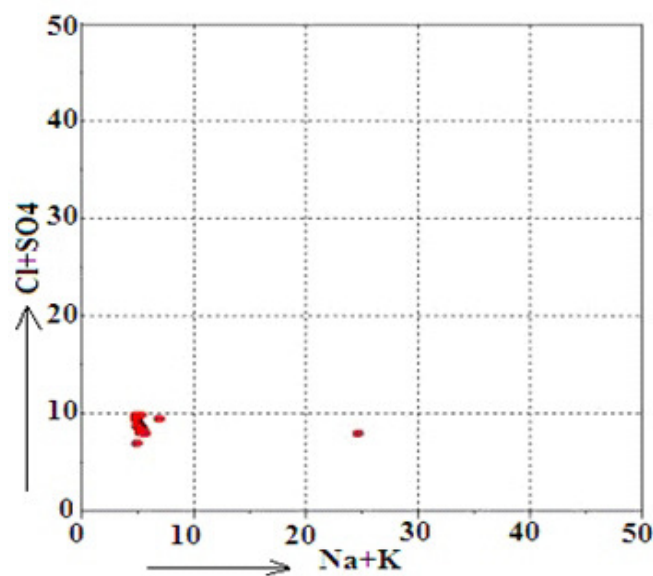


Figure.6. Langlier- Ludwig diagrams

Irrigation Quality assessment

The parameters such as sodium adsorption ratio (SAR), percent sodium (%Na) and residual sodium carbonate (RSC) were estimated to assess the suitability of water from the River for irrigation purpose. EC and sodium concentration are very important in classifying irrigation water. The total concentration of soluble salts in irrigation water can be expressed for the purpose of classification of irrigation water as low ($EC = <250$ IS

cm¹), medium (250750 IS cm¹), high (7502,250 IS cm¹) and very high (2,2505,000 IS cm¹) salinity zone (Richards 1954). While a high salt concentration (high EC) in water leads to formation of saline soil, a high sodium concentration leads to development of an alkaline soil. The sodium or alkali hazard in the use of water for irrigation is determined by the absolute and relative concentration of cations and is expressed in terms of SAR and it can be estimated by the formula:

$$SAR = \frac{Na^+}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}} \quad (\text{expressed in milli-equivalent per liter})$$

There is a significant relationship between SAR values of irrigation water and the extent to which sodium is absorbed by the soils. If water used for irrigation is high in sodium and low in calcium, the cation-exchange complex may become saturated with sodium. This can destroy the soil structure owing to dispersion of the clay particles. The plot of data on the US salinity diagram, in which the EC is taken as salinity hazard and SAR as alkalinity hazard, shows that the surface water samples fall in the category C1S1 and C2S1, indicating low to medium salinity and low sodium water which can be used for irrigation in most soil and crops with little danger of development of exchangeable sodium and salinity (Fig. 7). Sodium Percent is another parameter used to assess the suitability of water for irrigation and is calculated by formula

$$\%Na = (Na+K / Ca+Mg+Na+K) \times 100 \quad (\text{after Wilcox, 1955})$$

The sodium percentage (%Na) in the study area ranges between 17% and 21%. As per the BIS (Bureau of Indian Standard), maximum sodium of 60% is recommended for irrigation water. To quantify the effects of carbonate and bicarbonate, RSC has been computed. A high value of RSC (Residual Sodium Carbonate) in water values leads to an increase in the adsorption of sodium on soil (Eaton, 1950). Irrigation waters having RSC values greater than 5 meq/l have been considered harmful to the growth of plants,

while waters with RSC values above 2.5 meq/l is not considered suitable for irrigation purpose. The RSC values of the study area varied between 0.91.5 meq/l, again indicating that the water is safe for irrigation purposes.

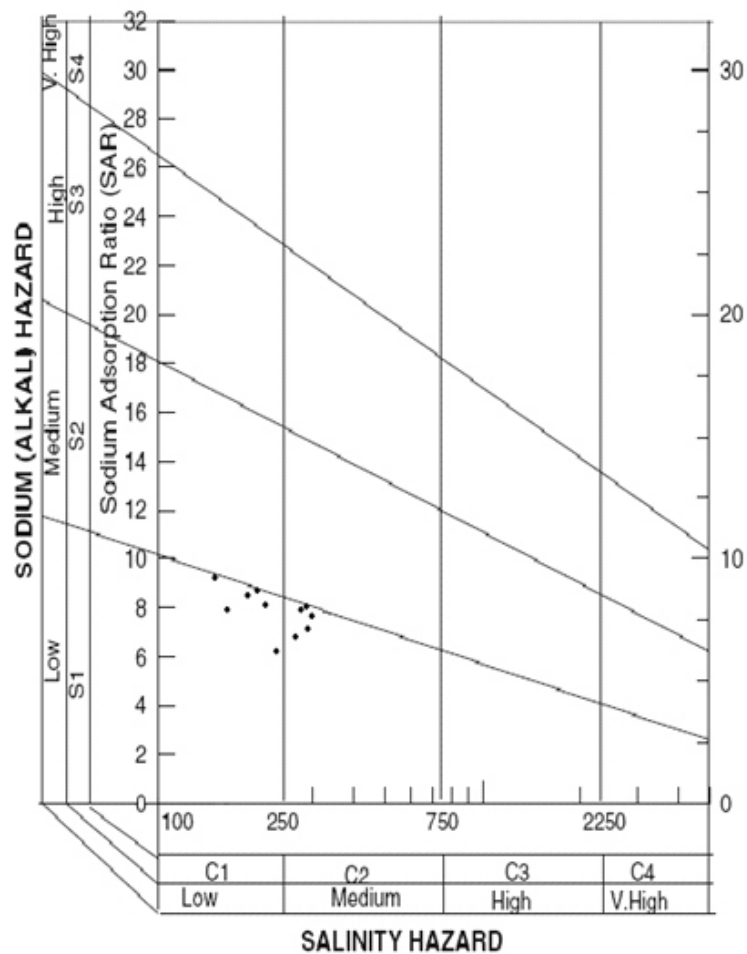


Fig.7. Salinity Hazard Diagram

CONCLUSIONS

From the forth going discussion, following conclusions were drawn.

- a) The water from Jhelum River is alkaline, medium electrical conductivity and total dissolved solids.
- b) The river water was found to be controlled by chemical weathering of the rock forming minerals; dominated by carbonates and silicates.
- c) Water of river Jhelum is meteoric, i.e; Ca-Mg-HCO₃.
- d) Analytical data from the study area confirm; river water present in the area is suitable for irrigation purposes.

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Exploring Antibacterial Activities of *Euphorbia* spp of Kashmir Against *Bovine mastitis*

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ABSTRACT

Bovine mastitis is the most significant economic drain on the worldwide dairy industry. Concerns regarding poor cure rates, emergence of bacterial resistance, and residues in milk necessitate development of alternative therapeutic approaches to conventional antibiotics for treatment of mastitis. New antibiotics and new therapeutic strategies are needed to address this challenge. Advances in identifying new sources of antibiotic natural products and expanding antibiotic chemical diversity are providing chemical leads for new drugs. Plants have traditionally provided a source of new chemical entities and numerous clinical studies have proved the therapeutic value of molecules of plant origin. The present study was conducted with the objective to evaluate the antibacterial activity of the hexane, methanol and aqueous extracts of selected medicinal plants against the microbes responsible for causing bovine mastitis. The hexane, methanol and aqueous extracts were obtained by extraction in cold maceration using hexane, methanol and water as solvents, respectively. The extracts were assessed for their antibacterial activity against *S.aureus* and *E.coli*, the two common pathogens responsible for causing mastitis. Out of the different extracts prepared only the methanolic and aqueous extracts of *Euphorbia* sp. were effective against *S.aureus*, the most important pathogen of bovine mastitis. The minimum inhibitory concentration (MIC) for the methanolic and aqueous extracts was found to be about 128 ug/ml and 256 ug/ml, respectively. The extracts of several other species of *Euphorbia* are under process.

Keywords: Bovine mastitis, antibacterial activity, mastitis pathogens, minimum inhibitor concentration (MIC), cold maceration, *Euphorbia* sp.

INTRODUCTION

Mastitis is an inflammation of the mammary glands of dairy cows that can be caused by physical or chemical agents, with the majority of cases caused by bacterial infection. Mastitis is the most common and expensive disease affecting the dairy industry worldwide (Harmon, 1994; Quinn *et al.*, 1994; Oussaoui *et al.*, 2004). The economic losses due to mastitis in the United States and worldwide have been estimated at US \$2 billion (Ott, 1999) and \$35 billion (Wellenberg *et al.*, 2002), respectively. In India, it is associated with an annual loss of about 7165.51 crores (Bansal and Gupta, 2009). The most common treatment method available for treating mastitis is the intramammary infusion of antibiotics. However, the cure rates obtained with antibiotics are generally poor and vary for different mastitis pathogens (Dingwell *et al.*, 2003). Further, the use of antibiotics may potentially lead to the emergence of antibiotic resistant strains of bacteria (Berghash *et al.*, 1983; White, 1999). Moreover, the use of antibiotics to treat bovine mastitis has been implicated as a common source of drug residues in milk (Erskine, 1996).. In light of the aforementioned problems and concerns, there is a need for alternative approaches for controlling mastitis in dairy cows.

Plants have traditionally provided a source of new chemical entities and numerous clinical studies have proved the therapeutic value of molecules of plant origin (Gibbons, 2004). Indeed, higher plant-derived products represent approximately 25% of drugs in current clinical use (Phillipson 2007). Of the more than 350 000 species of higher plants currently recognized, only 510% have been investigated and considering that each plant species may contain 500800 different secondary metabolites, the potential for the discovery of new therapeutic products in this largely untapped resource is considerable (Sibanda, *et al.*, 2007). The present study was undertaken to study the antibacterial effects of different extracts of *Euphorbia* sp. against important bovine mastitis pathogens.

MATERIALS AND METHODS

Plant collection. The plant material was collected from Gurez area of Kashmir Valley in the month of September 2013. It was properly identified with the help of experts.

Preparation of extracts: The collected plant material was separated into root and shoots and properly washed with tap water, air dried and pulverized into fine powder. Three different types of extracts namely hexane, methanol and water were prepared using the cold maceration technique. Briefly, the plant material was submerged in the respective solvents for 72 hours at room temperature with regular shaking and filtered through Whatman No 1 filter paper. The residue was macerated twice with the same solvent overnight and filtered. The filtrates obtained from each extraction were mixed and concentrated under vacuum. The extracts obtained were kept at 4 for further use.

Bacterial strains: The in vitro antimicrobial activities of the extracts of *Euphorbia sp.* were tested against *Staphylococcus aureus* and *Escherichia coli*, the two most common and important pathogens causing bovine mastitis in Kashmir Valley.

Antibacterial assay: Antimicrobial activities of the crude extracts were first screened for their inhibitory zone by the agar disc-diffusion method. Briefly, crude extracts were prepared at a concentration of 100mg/ml with dimethyl sulphoxide (DMSO) as solvent. The Mueller Hinton Agar (MHA) medium was used for disc diffusion assay and Mueller Hinton broth was used for the minimal inhibition concentration (MIC) determination. One hundred microliters (100 ul) of cell suspension with approximately 10^6 – 10^8 bacteria per milliliter was placed in petri dishes and dispersed over agar. In the following, a sterile paper disc (6 mm in diameter) impregnated with 10 ul of the plant extracts at the concentration of 100mg/ml and allowed to dry at 37 °C for 24 h was placed on to the agar and incubated overnight.

Minimum inhibitory concentration (MIC) determination: The minimum inhibitory concentration (MIC), which is considered as the lowest concentration of the sample

which inhibits the visible growth of a microbe was determined by the micro-broth dilution method. The MIC method was performed on extracts that showed their high efficacy against microorganisms by the disc diffusion method (inhibition zone higher than 11mm).

Table.1. Primary Screening of the samples against bacterial pathogens by disc diffusion assay.

S.No.	Tested Sample	Zone of inhibition (in mm)	
		S.aureus	E.coli
1	ELH	Inactive	Inactive
2	ELM	Active	Inactive
3	ELW	Active	Inactive
4	ERH	Inactive	Inactive
5	ERM	Active	Inactive
6	ERW	Active	Inactive
7	Ciprofloxacin	0.125	0.007

Table.2. MIC of the active sample by microdilution method

S.no	Compound	<i>S.aureus</i>
1	ERM	128
2	ERW	256
3	ELM	128
4	ELW	256
5	Ciprofloxacin	0.125

RESULTS AND DISCUSSION

In the present study, the in vitro antimicrobial activity of six extracts against two important microbial strains and their potential activity were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and MIC values. The zones of inhibition greater than 6mm were considered as positive. According to the

results given in Tables 12, the methanolic and aqueous extracts of the investigated plant species (i.e., *Euphorbia* sp.) showed in vitro antimicrobial activities against *S.aureus*, the most common and important pathogen causing bovine mastitis.

The results of this study provide a guide for further study of specific compounds in this plant particularly the methanol extract. Future work involves the purification and characterization of these compounds, determination of their antibacterial activity (MIC) against *S aureus* and to study their possible mechanism of action. The extracts of several other species of *Euphorbia* are under process.

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Man and Environment: an Opinion Analysis of Tourist Arrivals at a Destination

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ABSTRACT

This study helps to familiarize with the link between man and environment. The main objective of this study is to understand the meaning of Environment and its impact with man's intervention; it is depicted by scientists that tourist's arrival has vast impact on the destination through excessive usage of destination and degradation of environment. The data is collected from the nature based destination, Patnitop with the help of 5 point Likert scale and different statistical tools like mean, standard deviation are being used. The results depict that if carrying capacity is not employed at a destination it will definitely lead as environmental as well as destination degradation.

Keywords: Ecology, human beings, destination, tourists

INTRODUCTION

Environment can be defined as the means by which surroundings are understood and their upkeep maintenance and preservation are given prime importance. The environment is the complex set of physical, geographic, biological, social, cultural and political conditions that surround an individual or organism and ultimately determine its form and the nature of its survival. The environment

influences how people live and how societies develop. For that reason, people, progress, economic development and the environment are closely linked. It can be divided into non-living and living components. The Environment provides resources which support life on the earth and which also help in the growth of a relationship of interchange between living organisms and the environment in which they live. Human beings are the organisms having lot of effect on the environment of a destination as when there is mass tourism influx at a destination, the sustainability is compromised by their selfish needs. Tourism has a strong international dimension and is sensitive to any changes of climate that alter the competitive balance of holiday destinations. This change may be brought by the environmental degradation like global warming, ozone depletion and soil erosion. Furthermore, destinations which rely primarily upon their natural resource base to attract visitors, such as mountains and coasts are likely to be more at risk than those which depends upon cultural or historical attractions.

Tourism and recreation sector is highly influenced by climate and environment (Wall 1992, de Freitas 2003, Gomez-Martin 2005). Climate is an important factor in the destination choice of tourists (Maddison 2001, Lise and Tol 2002, Bigano *et al.*, 2007b, Bigano *et al.* 2008) and same is influenced by the number of factors excessive use of destination, accumulation of debris and misuse of resources. Tourism and the environment are continuously found in a relation of interdependence, as tourism is almost always dependent on the quality of the environment.

Tourism sector is highly influenced by climate, our understanding of how climate variability affects the sector and its potential vulnerability to climate change remains limited. Until recently, climate change had not garnered substantive attention from the tourism industry or the tourism and recreation research communities (Wall and Badke 1994, Scott *et al.* 2005 a; Gossling and Hall 2006). Due to the selfish needs of a

man there is a lot of destruction like killing of wild animals, deforestation which finally disturbs the eco system. This disturbance creates number of problems extinction of wildlife, loss of serenity of a destination and value of nature based tourist places.

Impacts of climate change domestic and international tourism and environmental impacts caused by domestic and international tourism are highlighted and the role of Environmental Education and sustainability as a response to all of these impacts is examined.

Strategic tourism assets, which are important for the sustainable growth of tourism in India, should be safeguarded from encroachment and damage by inappropriate development. These assets include special landscapes, important views, good water quality, the setting of historic buildings and monuments, biodiversity and access points to the coast and open countryside. The tourism industry is dependent upon the country's natural environment and cultural heritage to sustain the county's distinctive tourism product and to develop environmentally-based ecotourism products.

REVIEW OF LITERATURE

Tourism and the environment are continuously found in a relation of interdependence, as tourism is almost always dependent on the quality of the environment. Also, tourism today is deeply embedded in processes of global environmental change where natural scale and rate has dramatically increased because of human impact (Gossling and Hall, 2006). One factor that can potentially impact on perceived satisfaction is climate. Some destinations will see their peak season move away from the summer to the shoulder seasons and that destination at higher latitudes could experience a longer summer season. In contrast to all these, tourists' movement at a destination also perceived to be depended upon many factors (Jovičić, T., Jovičić, Ž. Ivanović, V., 2005):

- Appearance of accommodation capacity,
- Attractiveness of cultural and natural objects,
- Number of visitors,
- Transportation means used by visitors,
- Development of tourist trade management and environment management,
- Behavior of visitors and their awareness of the impact they leave on the environment and therefore their impact on the development of tourist offer in the area.

The entry of large number of tourists to nature areas could damage the natural resources in the areas. The environmental impact caused by tourism in protected areas and non-organized recreation activities, includes wildlife disturbance. If planning and development of tourism areas and tourism activities are not planned properly and controlled carefully, it can cause a decline in the quality of the environment, such as deterioration in the quality of water, air, noise and natural resources. But human greed for exploration and manipulation in the name of tourism, indirectly threaten the harmony and tranquility of the community of nature. To stop tourism's serious overexploitation of our nature and hereby prevent its destruction of exactly what it is based on, the relationship between tourism and ecology needs to be balanced by introducing the sustainability paradigm to tourism. Environmental decision-making process in tourism development is intrinsically complex and often involves multiple attributes, the relative importance of which needs to be determined. The preservation of natural and cultural values ensures that a particular destination will be attractive for tourists'. With this in mind, the study of tourism development and its impacts on the environment needs to be conceptualized at

different scales if a real progress towards a more sustainable development of tourism wants to be achieving (Hall, 1998).

TOURISM AND ENVIRONMENT

Tourism is an activity which is actually the composition of various elements like accommodation, transportation, destination services and facilities. These all are directly or indirectly depending upon the factors that influence environment like smoke producing transportation, waste produced by accommodation sector, debris and litter produced by tourist arrival at a destination. In order to keep track on these above cited activities. There is the high level involvement of man. So man should take such responsible steps in order to curb out the said menace and prevent the environment for their future generation. The tourism activity is human postured activity as it requires human brigade at every point of sale, it can be hotels, telephone booths, taxi drivers, shock holders at a destination etc. So in this way we can say tourism activities composed of tangible and intangible aspects during its execution. So, man is an important element in maintaining the sustainability and environmental responsibility. There is a lot of impact on the sustainability and responsibility of a destination if a tourist flow is not properly managed like due to excessive tourist flow at a destination can lead negative affects to the destination. That may vary from exploitation of flora and fauna to depletion of biodiversity. These two aspects of environment are necessarily to be managed in order to grab the concept of carrying capacity. This concept led a positive role in following number of measures like eco centricism, altruism and environmental preservation.

Research Objectives

1. To know about the man and environment interaction.
2. To know about the tourist opinion towards the effect on the destination.

3. To suggest the strategies for destination sustainability

Scope of the Study

This study is conducted at the famous tourist places of Jammu and Srinagar districts of J&K, in order to know the opinion of the tourist regarding the effect of tourist arrival on the ecology of a tourist destination. This paper is helpful tool for Destination Management Organizations (DMOs), researchers and academics to understand the tourist perspective about the effect of tourist on the ecology of a destination.

Research Methodology

This Paper is based on primary as well as secondary data as in this paper, the literature includes all the secondary data, while as quantitative portion is a primary data collected from the tourist travelling to Jammu and Kashmir regions with a total sample size of N= 130. The data was collected between Feb 2012 till March 2013.

Research Instrument

The self administered was designed to know the tourist perspective about the effect of various service providers on environment preservation. While as secondary data was collected from published journals, books and articles.

Research Techniques

In order to go for the descriptive analysis, the mean, standard deviation was applied. The data collection for this research was done after applying tools like 5- point Likert scale.

Table 1: Mean Score

S.No	Statement	Mean	S D
1	Trained Human Resource in tourism plays a positive role in sustainability	3.82	1.29
2	The excessive tourist flow affects the destination	3.76	0.99
3	Service providers at a destination can help in preventing the environment of the destination	3.87** *	1.13
4	Government has its positive impact on the environment preservation	3.51	1.04
5	Local transportation like horse riders can help in preserving the environment of the destination	3.51	1.23
6	Hotels can help in preserving an environment by using eco friendly products	3.87** *	1.09
7	Proper waste management techniques also help in preserving environment	4.33*	1.04
8	Banning polythene bags at a tourist place can also help in environment preservation	4.18**	1.20
9	Allowing a fixed number of tourist at a destination can save the destination	3.15	1.18
10	Concrete infrastructure at a destination has negative effect on the destination	3.13	1.11
11	Smoking at a destination also led harsh affect on the environment of the destination.	4.18**	0.90

Table 1, the mean values of all the statements asked, were taken and the highest mean value of 4.33 was given to the statement, "Proper waste management techniques also help in preserving environment", followed by "Banning polythene bags at a tourist place can also help in environment preservation", "Smoking at a destination also led harsh affect on the environment of the destination." (Mean value = 4.18) where as the lowest mean value of 3.13 was assigned to "Concrete infrastructure at a destination has negative effect on the destination"

SIGNIFICANCE OF THE STUDY

This research will help to create awareness among the tourists regarding the environment problem

CONCLUSION

The present study examined a tourist opinion about the effect of the tourist arrival on the ecology of a destination. A conceptual framework was proposed similar to what has been proposed in several previous studies establishing a link between . Overall, the investigation analyzed various factors like proper waste management techniques, banning of polythene & smoking at a tourist destination and the sensitization of various stakeholders regarding the preservation of environment at a tourist destination.

The current study has a number of limitations which suggest that further research into this aspect is necessary. First, the study has typically focused too narrowly on ecotourism destination, assuming that tourist who take an interest in nature and the environment impact the environment to a lesser extent than other tourists

Understanding the determinants could enable various destination management organization to adapt and improve their service, bringing their businesses in line with international standards. The study employs widely-used statistical tests, such as mean & standard deviation.

It is therefore evident from the results that in present times, majority of tourist visiting a tourism destination, are already sensitized about the environment and given a chance, they might switch to the environment friendly alternatives of polybags would prefer staying in a hotel which affect the environment in the minimum possible ways, use eco friendly modes of local transportation like ricksaw, tongas etc. In addition to this, it is clear from analysis that all the stakeholders from the local administration to the hoteliers, transport authorities, local community and tourist should work in synchronization to reduce the adverse effect of tourist on the host environment to the

minimum possible levels.

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Oral Health and Green Tea

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ABSTRACT

The Green tea is obtained from the tea plant *Camellia sinensis* (L.), Common names: Green tea extract, Chinese tea which belongs to the family *Theaceae*. Tea is the most consumed drink in the world after water. Green tea is a “non-fermented tea”, and contains more Catechins, than black tea or oolong tea. Catechins are in vitro and in vivo strong anti-oxidants. In addition, its content of certain minerals and vitamins increases the antioxidant potential of this type of tea. It is a widely used medicinal plant by the trials throughout India, China and popular in various indigenous system of medicine like Ayurveda, Unani and Homoeopathy Green tea has been consumed throughout the ages in India, China, Japan, and Thailand. Recent human studies suggest that green tea contributes to overall oral health. Green tea has been used in dentistry and has a promising role in future. This paper is an attempt to review potential role of green tea in promoting oral health.

Keywords: Green tea, *Camellia sinensis*, catechins, oral health

INTRODUCTION

Tea, a product made up from leaf and bud of the plant *Camellia sinensis*, is the second most consumed beverage in the world, well ahead of coffee, beer, and wine and carbonated soft drinks. Originating from India and China, tea has gained the world's taste in the past 2000 years. The economic and social interest of tea is clear and its

consumption is part of many people's daily routine, as an everyday drink and as a therapeutic aid in many illnesses (Wu and Wei 2002). There are three main varieties of tea—green, black, and Oolong. The difference between the tea's is in their Processing. Green tea is made from unfermented leaves and reportedly contains the highest concentration of powerful antioxidants called green tea poly phenols (GTP). The term —antioxidant refers to any molecule capable of scavenging or deactivating free radicalsdamaging compounds in the body that alter cells, tamper with DNA (genetic material) and even cause cell death. Free radicals occur naturally in the body, but environmental toxins (including ultraviolet rays from the sun, radiation, cigarette smoke, and air pollution) also give rise to these damaging particles. Scientists believe that free radicals contribute to the aging process as well as the development of a number of oral health problems including oral cancer (McKay and Blumberg, 2002). Antioxidants such as green tea poly phenols in green tea can neutralize free radicals and may reduce or even help prevent some of the damage they cause. Although health benefits have been attributed to green tea consumption since the beginning of its history, scientific investigations on this beverage and its constituents have been underway for less than three decades (McKay and Blumberg, 2002). Green tea poly phenols (GTP) may play a role in the risk and pathogenesis of several oral diseases, especially periodontal disease and oral cancer, and related pathologies. In addition, several studies suggest a beneficial impact of green tea intake on alveolar bone density and dental caries. The aim of this article is to revise the most recent studies on green tea's beneficial effects on oral health and to evaluate its potential interest.

Plant description and composition

Green, tea derived from the leaves of the *Camellia sinensis* plant. Originally cultivated in East Asia, this plant grows as large as a shrub or tree. Today, *Camellia sinensis* grows throughout Asia and parts of the Middle East and Africa. People in Asian countries more commonly consume green and oolong tea while black tea is most popular in the

United States. Green tea is prepared from unfermented leaves (McKay and Blumberg 2002). Green tea chemical composition is complex: proteins (1520% dry weight) whose enzymes constitute an important fraction; amino acids (14% dry weight) such as teanine or 5-N-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, lysine; carbohydrates (57% dry weight) such as cellulose, pectin's, glucose, fructose, sucrose; lipids as linoleic and linolenic acids; sterols as stigma sterol; vitamins (B, C, E); xanthic bases such as caffeine, theophylline, and pigments as chlorophyll and carotenoids; volatile compounds as aldehydes, alcohols, esters, lactones, hydrocarbons, etc.; minerals and trace elements (5% dry weight) such as Ca, Mg, Cr, Mn, Fe, Cu, Zn, Mo, Se, Na, P, Co, Sr, Ni, K, F Due to the great importance of the mineral presence in tea, the healthful properties of green tea are largely attributed to polyphenols, plant metabolites characterized by presence several phenol groups (i.e. aromatic rings with hydroxyls), which derive from Lphenylalanine (Stephano and Crispian, 2009) . The most important GTP's are tannins and flavonoids. The main flavonoids present in green tea include Catechins. The 4 major catechins are Epigallocatechin-3-gallate (EGCG) that represent approximately 59%of total catechins; epigallocatechin (EGC) (19% approximately); epicatechin-3-gallate (ECG) (13.6% approximately) and epicatechin (EC) (6.4% approximately) .EGCG is the most studied polyphenol component in green tea and the most active. Green tea also contains alkaloids including caffeine, bromine and theophylline (Carmen and Reyes 2006). These alkaloids provide Green's stimulant effects. The relative content of green tea Catechins depends on how the leaves are processed before fermentation. Wu and Wei indicated that a cup of green tea (2.5gms of green tea leaves/200ml of water) may contain 90mg of EGCG which is mainly responsible for its anti-oxidant, anti-carcinogenic, anti-inflammatory and anti-microbial properties.

Polyphenols in oral cavity

Direct antioxidant activity of poly phenols is valid in explaining their preventive effect

against diseases of the oral cavity where they come in direct contact with tissues before being absorbed and metabolized (Stephano and Crispian, 2009).

Polyphenol and oral cancer

The potential preventive activity of polyphenols against oral squamous cell carcinoma, the most common form of oral cancer is mainly because of Catechins. They inhibit the production of important metalloproteases (Ho *et al*, 2007), thus potentially reducing invasion and migration, inducing apoptosis and growth arrest in both oral cancer and oral leukoplakia cell lines (Lambert and Yang, 2003).

Polyphenols and periodontal disease

Inflammatory stimulation by periodontal pathogens increases the production of crevicular fluid and induces the chemotaxis of polymorphonuclear leukocytes, which, in order to inactivate periodontal pathogens, releases singlet oxygen and hypochlorous acid into the crevicular fluid. The consequent oxidative stress is countered by the antioxidant activity of ascorbate, albumin and urate present in the crevicular fluid and derived from plasma. However, this local oxidative stress may be increased by external factors or systemic conditions, such as smoking, diabetes, obesity and metabolic syndrome. When there is disequilibrium between oxidative stress and antioxidant activity, periodontal tissue destruction may appear. These observations suggest that antioxidant rich diets might inhibit periodontal disease development and progression, particularly in subjects exposed to environmental and dietary sources of oxidative stress (Battino and Bullon, 1999). Several studies also report that decreased antioxidant activities of crevicular fluid and saliva are associated with the development of periodontitis (Ritchie and Kinane, 2003). GTPs may contribute to increase the antioxidant activity of oral fluids. Delivery of GTPs by holding green tea in the mouth for 25 min increases the antioxidant capacity of saliva, and daily consumption of two cups of green tea for 2 weeks increases the phagocytic capacity of the polymorphonuclear leucocytes in the gingival crevicular fluid (Lee and Lambert, 2004). Green tea catechins,

used in a slow-release local delivery strip system applied in the periodontal pockets, decrease the pocket depth and the proportion of Gram negative anaerobic rods, while the same catechins show an in vitro bactericidal effect against *Porphyromonas gingivalis* and *Prevotella* sp. Several GTPs inhibit the proteolytic activity of *Porphyromonas gingivalis*. In addition, GTPs counteract the production of prostaglandin E2 induced by *Porphyromonas gingivalis* (Hirasawa and Takada, 2002).

Polyphenils and dental caries

Dental caries is a multi-factorial infectious disease in which nutrition, microbiological infection, and host response play important roles. Earlier reports in experimental animals and humans suggested that green tea consumption (without added sugar) reduces dental caries (Elvin-Lewis and Vitale, 1980). Linke and LeGeros (Linke and LeGeros, 2003) indicated that frequent intake of green tea can significantly decrease caries formation, even in the presence of sugars in the diet. *In vivo* animal studies have shown that specific pathogen free rats infected with *Streptococcus mutans* and then fed with a carcinogenic diet containing GTP have significantly lower caries scores (Otake and Makimura, 1991). Supplementing drinking water of rats with 0.1% GTP along with a cariogenic diet also significantly reduced total fissure caries lesions. Recent findings of Okamoto et al (Okamoto and Sugimoto, 2004) suggest that green tea catechins may have the potential to reduce periodontal breakdown resulting from the potent proteinase activity of *Porphyromonas gingivalis*. In addition, green tea decoctions inhibit alpha-amylase in human saliva, reducing maltose release by 70% and effectively lowering the cariogenic potential of starch containing food. Similarly, Zhang and Kashket (Zhang and Kashket, 1998) reported that green tea extracts inhibits human salivary amylase and may reduce the cariogenic potential of starch-containing food such as crackers and cakes because it may reduce the tendency of this kind of food to serve as slow-release sources of fermentable carbohydrate. It is likely that the cariogenic challenge in a cariogenic diet may be reduced by the

simultaneous presence of green tea in the diet. Apart from their polyphenol content green tea, is a natural source of fluoride and an effective vehicle for fluoride delivery to the oral cavity. According to Simpson et al (Simpson and Shaw 2001), after cleansing the mouth with tea, approximately 34% of the fluoride is retained and shows a strong binding ability to interact with the oral tissues and their surface integuments. This fluoride content may have a beneficial impact on caries and may carry out a wide range of biological activities including prevention of tooth loss and oral cancer (Lambert and Yang, 2003). Nonetheless, the data have suggested that GTP extract may be responsible for the noted effects on oral health and it has been also demonstrated that GTP rather than fluoride contribute to anti-cariogenic potential by inhibition of oral bacteria growth such as *Escherichia coli*, *Streptococcus salivarius*, and *Streptococcus mutans*. Several studies have indicated that GTP inhibits growth, acid production, metabolism, and glucosyltransferase enzyme activity of *S. mutans* and dental plaque bacteria. Thus, green tea has been considered as functional food for oral health and is widely used in toothpaste formulation. Poly phenols and root canal flora (Horiba *et al*, 1991) studied the antibacterial and bactericidal effects of green tea as an intracanal medicament on different bacterial strains and found that extracts of Japanese green tea may be useful as a medicament for treatment of infected root canals. Prabhakar *et al*, (2010), showed statistically significant antibacterial activity against *E. faecalis* biofilm formed on tooth substrate and demonstrated that it takes 6 minutes to achieve 100% killing of *E. faecalis*.

CONCLUSION

Green tea has been consumed in China and other Asian countries since ancient times in order to maintain and improve health. Nowadays, green tea is considered one of the most promising dietary agents for the prevention and treatment of many oral diseases and consequently, it is being studied extensively worldwide. Numerous studies in a variety of experimental animal models have demonstrated that aqueous extract of the major GTP designated as Catechins (EGCG, EGC, ECG and EC) possess antioxidant, antimutagenic, antidiabetic, antiinflammatory, antibacterial and antiviral, and above all, cancer-preventive properties. Epidemiological studies suggest that consumption of green tea may have a protective effect against the development of oral cancer. Preclinical studies of green tea and its polyphenolic components have demonstrated antimutagenic and anticarcinogenic activity, and inhibition of growth of tumor cell lines and animal tumor models, including oral cancer. In addition, several epidemiological studies with humans have demonstrated that regular green tea consumption has beneficial effects and it shows a significant rate of protection against the development of dental caries and many periodontal diseases. It also contributes to body weight control and to the rise of bone density as well as being able to stimulate the immune system. Catechin antioxidant power is also strengthened by the presence of other phenolic compounds, vitamin C and minerals such as Cr, Mn, Se, and Zn, although specific data regarding this fact are still scarce. However, conflicting results between cohort studies conducted in different countries may also arise from confusion in the frequency and timing of intake, and the marked contrasts in the socioeconomic and lifestyle factors associated with tea drinkers. It is also important to consider the type of tea or its preparation (e.g., short time vs. long brewing time and hot tea vs. iced tea) due to the marked impact of these factors on poly phenol content and concentration. It is also important to draw attention on the need of further-in-depth

studies on the nature and mechanisms of the active green tea compounds, on the bioavailability of the different Catechins in humans, and appropriate dose levels to act as functional food. Since green tea beneficial oral health effects are being increasingly proved, it could be advisable to encourage the regular consumption of this widely available, tasty and inexpensive beverage as an interesting alternative to other drinks, which do not only show the beneficial effects, but are also more energetic, do contain caffeine (green tea contains less caffeine than black tea, coffee or cola soft-drinks), are rich in additives and/or CO₂. While no single food item can be expected to provide a significant effect on oral health, it is important to note that a modest effect between a dietary component and a disease having a major impact on the most prevalent causes of morbidity and mortality, i.e., oral cancer should be given substantial attention. Taking all this into account, it would be advisable to consider the regular consumption of green tea in diet.

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Hydrogeological Scenario in Kashmir Valley with Special Reference to Water Level and Water Quality of Phreatic Aquifer

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ABSTRACT

The present paper discusses the changing hydrogeological scenario in Kashmir Valley & its impact on ground water regime. The paper also discusses the regional behavior of water levels in phreatic aquifers for the period of May 2009 and November 2009 with seasonal fluctuation. The results of the chemical analysis of water samples collected in May, indicates that the water is fresh and potable. The result of chemical analysis of phreatic (shallow) aquifer is also discussed

Keywords: Phreatic aquifer, NHNS, water level, fluctuation.

INTRODUCTION OF STUDY AREA

Geology

Lying between the longitudes 33025'E and 34032'E and latitudes 7400'E and 75030'E, the high altitude valley of Kashmir is an ovoid basin with a nearly flat floor of around 4920 km² and is existing between the lesser and greater Himalayas. The vale of Kashmir with tectonic origin is 135 km long and 45 km broad at its middle, lying as an oval bowl between the Zanaskar range to the North and Pir Panjal range to the South. Most of the valley lies at an elevation of just over 1500 m, though its floor rises steadily from northwest to southwest (Wadia, 1966). The tectono-geomorphic setting of the Kashmir Valley reveals that due to rise of the Pir-Panjal Range, the primeval drainage was impounded as a vast lake in which the sediments of Karewa Group were deposited as intermontane valley fill deposits (Dar *et al*, 2013). Kashmir Valley is an

intermountain valley fill, comprising unconsolidated gravel sand and mud succession known as Karewas divided into two stages, lower and upper, representing argillaceous and arenaceous facies respectively.

Geologically the strata that compose the low lying areas of kashmir valley are favorable for the occurrence of groundwater (Ahmed and Ahmed, 2013). This formation of Plio-Pleistocene age lies discomformably over the older rocks ranging in age from Cambrian to Triassic.

Formation	Age
Alluvium	Sub Recent -Recent
rewash	Plio-Pleistocene
Lime stone	Triassic
Panjal Volcanics	Paleozoic
Agglomeratic Slate Series	Late Carboniferous to early Permian
Muth Quartzites	Late Silurian to Early Devonian

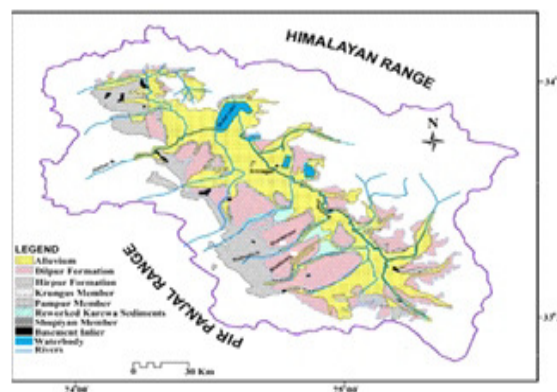


Figure1: Geological map showing distribution of Karewa Group of Sediments modified after Bhatt, 1982

Hydrogeology

Kashmir valley has rich deposits of ground water in both confined and unconfined aquifer system, but its occurrence is highly uneven due to diverse geological formations (Singh and Sharma, 1999). Despite its vastness and significance, groundwater in Kashmir Valley has received very little attention regarding estimation of quality, quantity, conservation and management (Jehangir *et. al*, 2011). Hydrogeologically, the Geological formations of Kashmir valley can be classified into two categories (GWIB, 2009)

- a. Hard or consolidated formation: Hard or consolidated formations comprising of granites, slates, quartzites, traps, limestones etc belonging older than tertiary age. Ground water in consolidated formation occurs in secondary porosity in the form of fractures and joints. Yield potentiality of these formation is very limited with varying discharges, because of highly jointed and fractured nature of these formations, these area form very good recharge zones and recharge the aquifers underlying the Karewas formation in valley areas.
- b. Unconsolidated formation: Unconsolidated formation comprising of clay, silt, sand, gravels and boulders etc belonging quaternary to recent in age. The Karewas Basin in the Kashmir Himalayan preserves a record of sediment fill in an intermontane basin (lake) formed during the Late Neocene to Quaternary period in which the sedimentation is controlled the tectonic events. The main water bearing horizons in Kashmir basin are mainly Karewa succession except a marginal area of piedmont zone along the hills. The aquifer in Upper Karewa formations and at few places thin sand horizons and boulders occurring in Lower Karewas formations are main water aquifers in the area, Discharge of tube wells generally ranges from 238 LPM to 4164 LPM

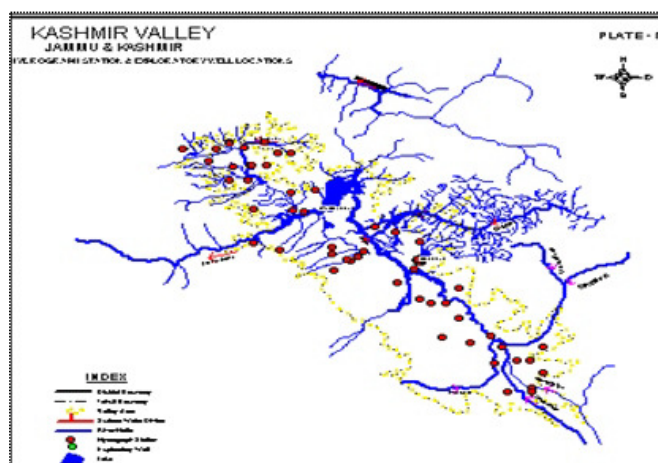


Figure 2: Kashmir valley map showing drainage and Monitoring/sampling locations

METHODOLOGY

Twenty five hydrograph stations were monitored and samples were collected from the phreatic aquifer during pre monsoon period of 2009, all across the Kashmir Valley in pre-rinsed HDPE bottles (Scaif, *et al.*, 1987). Water samples were filtered with 42 μm filters and collected in two sets. One set was immediately acidified with concentrated HNO_3 to maintain the pH of the samples. All the chemical constituents were analysed following standard methods (APHA1995). Field parameters like Temperature, electrical conductivity (EC) and pH were measured in field itself using a potable water analysis kit. Carbonate and Bicarbonate are determined titrimetrically (acid base titration). Chloride is determined by titrating with silver nitrate. Total Hardness, Calcium and Magnesium are determined titrimetrically using standard EDTA solution. Sulphate is determined by using turbidity meter. Nitrate and Fluoride are estimated by using Spectrophotometer. The alkali metals Sodium and Potassium are determined by Flame photometer (Systronics).

The water level data of monitoring stations Kashmir Valley was analyzed using the

dedicated software 'GEMS' and water level contours were plotted by using MapInfo & Vertical Mapper software by 'natural neighborhood' interpolation method. The map shows the spatial and temporal variations of the parameters in the area.

DISCUSSIONS

Ground Water Behavior

The water level behavior in the Kashmir valley is entirely different from the other parts of the country. This is mainly because of the fact that 60 to 70% of the precipitation is received in the form of snow during December to February while March & April are the months of heavy rainfall. May to September are relatively dry months. Hence recharge to the ground water takes place in the valley in the months of April to June with melting of snow and with the beginning of rainfall. Therefore water level shows trends of rising from April (summer) onwards and falling from August onwards.

Table1: Water level data of NHS wells with fluctuation

S.No	Name of the well	May-09	Nov-09	Fluctuation
1	Sambura	3.42	6.12	2.7
2	Pampora	6.5	6.16	-0.34
3	Zeewan	4.13	4.62	0.49
4	Regal Chowk	2.81	5.67	2.86
5	Mirgund	1.6	3.85	2.25
6	Sangrama	1.33	2.09	0.76
7	Udipora	1.26	4.35	3.09
8	Langyt	1.65	7.83	6.18
9	Chowgal	1.16	4.34	3.18
10	Sopor	0.64	2.2	1.56
11	Azmathpora	0.6	1.39	0.79
12	Bomai	1.21	2.62	1.41
13	Gulgam	2.77	4.21	1.44
14	Kupwara	2.71	5.41	2.7
15	Drugmulla village	1.6	4.41	2.81
16	Panzgam	1.98	3.2	1.22
17	Dolipur	1.6	4.6	3
18	Magam	1.9	4.85	2.95
19	Trehgam	2.8	6.46	3.66
20	Warsu	4.57	5.2	0.63

In pre-monsoon (May 2009), about 57% of the total hydrograph stations come in the water level range of 0-2 m bgl. About 28% comes in the water level range of 2-5 m bgl, 4% in the range of 5-10m , 10-20m and >20 m bgl. The data is shown the thematic layer as below.

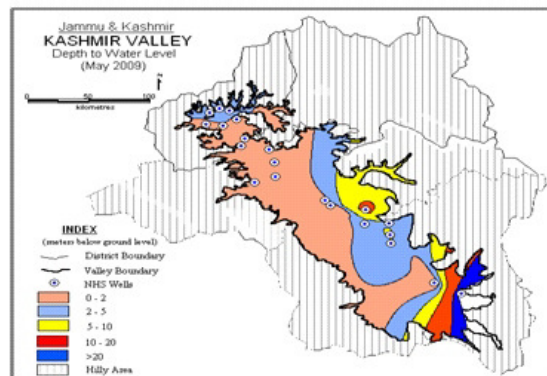


Figure 3: Kashmir valley map showing DTW in May 2009

In post monsoon (November 2009), about 2% of the total hydrograph stations come in the water level range of 0-2 m bgl. About 11% comes in the water level range of 2-5 m bgl, 6% in the range of 5-10m and 2% comes in the range of 10-20m bgl. The data is shown the thematic layer as below.

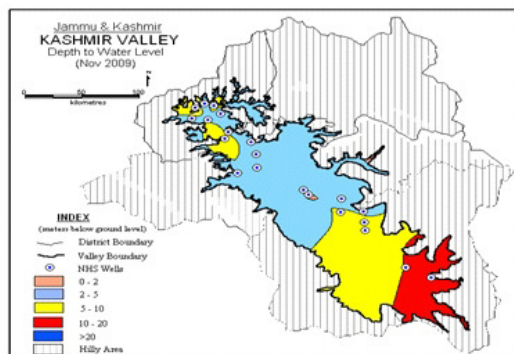


Figure 4: Kashmir valley map showing DTW in November 2009

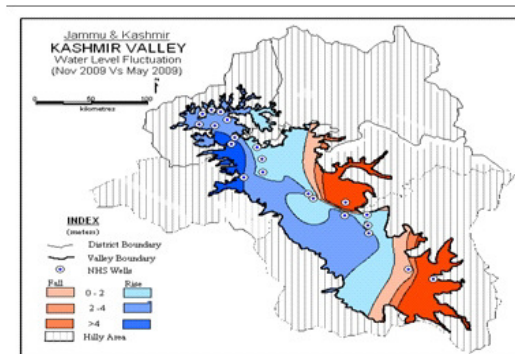


Figure 5: Kashmir valley map showing Fluctuation

Chemical Behavior

For interpretation of Hydrochemistry of Kashmir valley, water sample collected from shallow aquifer and analyzed. Water quality parameters of shallow aquifer are summarized in table given below.

Table 2: Water level data of NHS wells with fluctuation

S. No.	Water Quality Parameters	Location	
		Minimum	Maximum
1	pH	6.9 Kangan (Pulwama)	8.80 Kadalbal Sufi Mohlla
2	Electrical conductivity $\mu\text{mhos/cm}$ at 25°C	97 Udipora (Kupwara)	2800 Pampore (Pulwama)
3	Bi carbonate (mg/l)	49 Udipora (Kupwara)	708 Gundemacher(Kupwara)
4	Chloride (mg/l)	04 Dholipora(Baramulla)	273 Pampore (Pulwama)
5	Nitrate (mg/l)	0.48 Regal Chowk (Srinagar)	394 Pampore (Pulwama)
6	Fluoride (mg/l)	0.01 Regal Chowk (Srinagar)	1.00 Malingpur (Anantnag)
7	Sulphate (mg/l)	02 Sodipura (Anantnag)	260 Pampore (Pulwama)
8	Calcium (mg/l)	12 Udipora (Kupwara)	174 Gundemacher(Kupwara)
9	Magnesium (mg/l)	2.6 Rambelpur (Anantnag)	193 Pampore (Pulwama)
10	Sodium (mg/l)	2.0 Udipora (Kupwara)	160 Pampore (Pulwama)
11	Potassium (mg/l)	0.2 Telbal (Srinagar)	215 Kadalbal Sufi Mohlla
12	Total Hardness as CaCO_3 (mg/l)	42 Udipora (Kupwara)	871 Pampore (Pulwama)
13	Iron (mg/l)	0.03 Zakura (Srinagar)	5.72 Sadipur (Pulwama)

RESULTS

Hydrogeology

- The water level data from phreatic aquifers reveals that the water levels are shallow in the month of May. Deeper water levels are reported in the month of November.

- Water level ranges from 0.6m bgl (in Azmathpora) to 6.5 m bgl (in Pampora) in the month of May 2009.
- Similarly Water level ranges from 1.39m bgl (in Azmathpora) to 7.83m bgl (in Langyt) in the month of November 2009.
- As the water levels are very shallow, most of the water levels are shown in the range of 0-2m bgl in May 2009. 12 numbers of wells falls in 0-2m bgl, 7 wells in 2- 5m bgl and 1 well in the range of 5-10m bgl.
- In November, as the water levels have gone down, 1 well falls in the range of 0- 2m bgl, 12 wells fall in the range of 2-5m bgl and 7 wells fall in the range of 5-10m bgl.
- When the water level data for May 2009 was compared with November 09, it was revealed that 8 wells have shown rise in water level from 0-2m, 10 wells have shown rise from 2-4 m and only 1 well have shown rise more than 4m.

Hydrochemistry

- The chemical analysis shows that the water is fresh and potable.
- The value of pH shows that shallow ground water is alkaline in nature. Its ranges from 6.9 (Kangan) to 8.80 (Kadalbal Sufi Mohlla).
- Electrical conductivity value ranges from 97 μ mhos/cm (Udipora) to 2800 μ mhos/cm (Pampore).
- Bi-carbonate value ranges from 49mg/l (Udipora) to 708mg/l (Gundemacher).
- Chloride value ranges from 04mg/l (Dholipora) to 273mg/l (Pampore).
- Nitrate value ranges from 0.4mg/l (Regal Chowk) to 394mg/l (Pampore) and shows that Pampore's shallow water exceeding the permissible limit. For nitrate permissible limit is 45 mg/l for drinking water standard set up by BIS.
- Fluoride value ranges from 0.01mg/l (Regal Chowk) to 1.00mg/l (Malingpur) and

shows that concentration of fluoride is within permissible limit (1.5 mg/l) for drinking purpose (BIS)

- Sulphate value ranges from 02 mg/l (Sodipura) to 260mg/l (Pampore) and shows that the concentration of sulphate in shallow aquifer is within the maximum permeable limit (400 mg/l)
- Calcium value ranges from 12mg/l (Udipora) to 174mg/l (Gundemacher).
- Magnesium value ranges from 2.6 mg/l (Rambelpur) to 193mg/l (Pampore).
- Sodium value ranges from 2.0 mg/l (Udipora) to 160mg/l (Pampore).
- Potassium is also important parameter for quality aspect. In shallow aquifer potassium value ranges from 0.2mg/l (Telbal) to 215mg/l (Kadalbal Sufi Mohlla).
- Harness of water is the capacity to neutralize soap and is caused by carbonate and bicarbonate of calcium, magnesium. In shallow aquifer total hardness value ranges from 42mg/l (Udipora) to 871mg/l (Pampore).
- Iron value ranges from 0.03mg/l (Zakura) to 5.72mg/l (Sadipur) and shows that the maximum concentration of iron 5.72 mg/l (Sadipur) is exceeding the maximum permissible limit (1.0 mg/l) of BIS for drinking water purposes.

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