REVIEW OF LITERATURE

DEVELOPMENT AND DIFFERENTIATION OF PROTONEMA

In the life cycle of bryophytes, two distinct phases occur and these two phases alternate with each other. These are known as gametophytic and sporophytic phase. Gametophytic phase starts with formation of spore which on germination produces protonema. Müller (1874) while studying the protonema of Funaria hygrometrica distinguished two types of filaments. The first type of filaments extends over the substratum and are composed of cells having abundant chlorophyll. The filaments of second type grow downwardly into the substratum and are pale or brownish rhizoid-like structures. He opined that these two types of protonema are morphologically homologous and can undergo transformation into the other. Similar observations were made by Sachs (1875) and Goebel (1882). Correns (1899) coined the terms chloronema and caulonema for these two types of filaments of protonema. He used the term chloronema for filaments with abundant chlorophyll, hyaline cell walls and perpendicular cross walls, whereas the term caulonema was used for filaments with brownish cell walls and oblique cross walls. He also observed many transitions between these two forms and formation of chloronema from rhizoids when these are detached from the gametophyte. Woesterdijk (1907) observed that in the chloronema, branches of successive order are of the same width, whereas branches of rhizoids become progressively narrower. The importance of light in differentiation of protonemal filaments into chloronema and rhizoids was recognized by Müller (1874) and Sachs (1875). Goebel (1905) and Woesterdijk (1907) reported that rhizoids can produce chloronema only when they were damaged or when the shoot apex is removed. It was considered a correlative phenomenon. Servettaz (1913) also reported two stages in the development of protonema: protonema rampant and protonema dresse. In first stage, “protonema rampant” protonemal filaments spread over the substratum. In second stage, “protonema dresse” vertical aerial filaments develop from the first stage. Woesler (1933) also recognized existence of aerial and prostrate filaments.

Sironval (1947) observed that in Funaria hygrometrica protonema comprises of two distinct stages: chloronema and caulonema. The first stage or chloronema
resulting from germination of spore, is green, filamentous, poor and irregularly branched, photopositive structure. According to him, all the cells except apical cells of chloronema degenerated after about three weeks and the persistent apical cells gave rise to next stage, caulonema. Filaments of caulonema are capable of producing gametophores. Caulonemal filaments have brownish cell walls, oblique cross walls and poor in chlorophyll. However, branches of caulonema are like chloronema. Van Andel (1952); Bopp (1954, 1955, 1959 a,b, 1961). Allsopp and Mitra (1956, 1958) and Kofler (1959) accepted the terms chloronema and caulonema but they did not agree with Sironval (1947) about the mode of caulonema development. According to them, the differentiation of caulonema is a continuous process. Kofler (1956) reported that in *Funaria hygrometrica*, appearance and extent of caulonema formation is largely dependent on culture conditions as well as composition of the medium. According to her, caulonema is not an obligatory phase of development that must be reached prior to bud initiation. Allsopp and Mitra (1958) studied protonemal development in 15 species belonging to 14 genera: *Atrichum undulatum, Brachythecium rutabulum, Bryum capillare, Ceratodon purpureus, Cinclidotus fontinaloides, Dicranella heteromala, Eurhynchium riparioides, Funaria hygrometrica, Grimmia pulvinata, Leptobryum pyriforme, Mnium hornum, Pleuridium acuminatum, Pohlia nutans, Tortula muralis* and *T. subulata*. No sharp distinction between chloronema and caulonema was observed. Protonema showed heterotrichous habit with prostrate filaments of relatively unlimited growth resembling caulonema and erect filaments of more restricted growth resembling typical chloronema. Nebel and Naylor (1968a) failed to observe stage comparable to chloronema in the moss *Physcomitrium turbinatum*. Differentiation of protonema into typical chloronema and caulonema with a heterotrichous habit has been observed in *Physcomitrium coorgense* (Lal, 1961), *Anoectangium thomsonii* (Rashid, 1970), *Timmiella anomala* (Chopra & Rekhi, 1979), *Barbula gregaria, Dicranella coarctata, Racomitrium lanuginosum* (Kumra, 1981), *Bartramidula bartramiioides* (Chopra & Rahbar, 1982), *Anisotheicum molliculum* (Kumra & Chopra, 1985), *Trematodon brevicalyx, Philonotis lancifolia* (Babbar, 1985), *Garckea phascoides, Bryum pallescens* (Sarla, 1986), *Anisotheicum spirale, Anoectangium strachyanum,*
*Didymodon recurvus* (Vashistha & Chopra, 1987) and *Microdus brasiliensis* (Chopra & Mehta, 1987)

Involvement of two steps in the differentiation of moss protonema is also substantiated by X-ray induced mutants (Oehlkers, 1956; Hatanaka-Ernst, 1966) and auxin-resistant mutants of *Funaria hygrometrica* (Bhatla & Bopp, 1985). The protonema of these mutants remain arrested at the chloronema stage suggesting two steps, chloronema and caulonema in the differentiation of protonema. Caulonema formation does not occur under unfavorable conditions such as low light intensity (Bopp, 1959 a,b; Lehnert, 1982), low temperature (Bopp, 1959a), liquid culture (Szweykowska, 1962; Johri, 1974) and on soft agar (Kofler, 1959). Caulonema dedifferentiates into chloronema when subjected to unfavourable conditions (Knoop, 1973; Bopp, 1980) or on isolation and subculture on similar medium (Bopp & Böhrs 1965).

Many bryologists suggested that protonemal differentiation is regulated by some endogenous morphoregulatory substances (Bopp, 1963; Bopp & Klein, 1963; Jahn, 1964; Klein, 1967). These workers reported that the chloronemal cells of *Funaria hygrometrica* produce, a heat liable factor ‘F’ which promotes the differentiation of caulonema from chloronema but inhibits bud initiation. Caulonema cells synthesize a heat-stable factor ‘H’ which at higher concentrations promotes bud initiation and inhibits caulonema growth. Rawat and Chopra (1976) recognized factor ‘H’ like thermo stable factor in *Bryum klinggraeffii*. This factor was inhibitory for protonemal growth but promoted gemma formation.

Nishida (1978) and Nehira (1983, 1988) found protonemal growth patterns on mosses constant for each taxon and are unrelated to the culture conditions. Contrary to this, Alcalde *et al.* (1996) observed that variations in protonema morphology in *Bartramia* can be induced by changing variables such as temperature, photoperiod, physical state and nutritional composition of the media.

Protonemal differentiation involves certain chemical factors. Kofler (1959) reported that in *Funaria hygrometrica* the growth of chloronema was inhibited at higher levels of auxins. Auxins favour caulonema differentiation and inhibit growth of chloronema (Bopp, 1963; Hurel-Py, 1948; Johri & Desai, 1973; Szweykowska, 1962).
Johri (1974) found that NAA was ineffective in caulonema differentiation. Bopp (1963) and Cove et al. (1979) reported that protonemal differentiation is regulated by the substance released by the developing protonema. These substances include hormones as well as enzymes (Knoop, 1973). The effect of auxin depends on its concentration and density of inoculum. The density effect is a function of release of IAA-oxidase into the medium which degrades IAA present in the medium. Activity of IAA-oxidase is highest in high density cultures and due to this at high inoculum density, higher auxin level is required for a change of chloronema to caulonema (Johri, 1978; Sharma et al., 1979; Johri & D’Souza, 1990). Bopp (1980) reported that auxins IAA, 2,4-D and NAA induce caulonema formation up to the same percentage as observed in high light intensity without auxin. According to Lehnert and Bopp (1983), intermediates of the main auxin synthesis pathway i.e. tryptophan, indoleacetaldehyde and indolepyruvic acid are effective in promoting caulonema formation, whereas tryptamine and indoleacetonitrile are ineffective. Auxin antagonist like PCIB (Bopp, 1979, 1980; Sood & Hackenberg, 1979) and inhibitors of photosynthesis like DCMU (Knoop, 1973) cause dedifferentiation of caulonema into chloronema suggesting definite role of endogenous auxin content in protonemal differentiation. This fact was supported by experiments with mutants of Physcomitrella patens (Ashton & Cove, 1977; Ashton et al., 1979 a,b; Cove et al. 1979). Atzorn et al. (1989b) demonstrated involvement of endogenous IAA in regulating protonema differentiation by estimation of endogenous IAA levels in the wild type and certain auxin-sensitive mutants of Funaria hygrometrica. HPLC-ELISA determination of endogenous IAA after tryptophan application in an auxin-insensitive mutant (86-1) of the moss F. hygrometrica revealed a steady increase in endogenous IAA level with age clearly demonstrated that tryptophan uptake and tryptophan metabolizing systems are operational. But inability of caulonema formation even by tryptophan treatment shows that there may be disruption of signal chain of IAA reaction system (Bhatla & Bopp, 1992). The maintenance of the chloronema state and caulonema differentiation has been observed to be regulated by cyclic 3’, 5’-AMP and IAA respectively (Atzorn et al., 1989 a,b; Bhatla, 1994; Bopp & Atzorn, 1992; Handa & Johri, 1976, 1979). According to Handa and Johri (1977), endogenous concentration of cAMP is 4-7 times higher in chloronema than that in caulonema and
a high cAMP/IAA ratio (20) leads to chloronema formation. Other nucleotides have also been observed to increase proportion of chloronema (Handa & Johri, 1979). Earlier Johri and Desai (1973) also emphasized the role of cAMP in protonema differentiation by using cyclic nucleotide phosphodiesterases (cNDPE), which increases cAMP, IAA oxidase and peroxidase. In contrast, Kaul and Sachar (1982) failed to detect cAMP in *F. hygrometrica*. Kapoor and Bhatla (1996) using auxin-sensitive and auxin-insensitive mutants of *Funaria* observed protonemal differentiation to be governed by an interaction of auxin, cAMP, ethylene and calcium. The role of auxin in protonemal differentiation is both inductive (mutant 87.13 and NAR-2) and stimulatory (Wild type). The role of precursor of ethylene biosynthesis i.e. l-aminocyclopropane l-carboxylic acid (ACC) is also similar. Increase in amount of ACC and ethylene during protonemal growth shows its involvement in chloronemal differentiation. Interaction of auxin with ethylene and cAMP appears to act through an elevation in the intracellular Ca\(^{2+}\) concentration as demonstrated by use of Ca\(^{2+}\) ionophore A 23187 (Kapoor & Bhatla, 1996).

Menon and Lal (1974) in *Physcomitrium*, and Lehnert and Bopp (1983) in *Funaria* observed that ABA caused formation of shorter cells. Bopp and Coworkers (Bopp & Werner 1993, Werner & Bopp 1993) have shown that exogenously applied ABA in *Funaria* protonema induces synthesis of several proteins and also influences protein phosphorylation. ABA induces moss chloronemata to differentiate multicellular or unicellular propagules of short cells known as brachycytes. Goode et al. (1993a) used the term brood cell. Caulonemata become chloronematic before they redifferentiate into brachycytes (Goode et al. 1993b). The number of developing brachycytes depends on ABA concentration and the duration of treatment. Brood cells or brachycytes are propagules capable of survival and dissemination. ABA induces tmema cells formation in auxin deficient mutant 87.25. Actually ABA has qualitative and quantitative effect on brachycyte differentiation (Schnepf & Reinhardt, 1997).
BUD INITIATION

Bud induction on caulonema is the next important development stage in the life cycle of a moss. This fundamental step results in the transition from one dimensional filament extension to three-dimensional gametophore growth. This transition in growth pattern is associated with changes in plane and rate of cell division, deposition of wall material (Bopp & Fell, 1966), structure of chloroplast and orientation of microtubule (Mlodzianowski & Szweykowska, 1971; De Maggio & Stetler, 1977; Idzikowska & Szweykowska, 1978).

In Funaria, buds are initiated when the protonema attains a certain critical size (Bopp & Brandes, 1964). In Bryum klinggraeffii (Rawat & Chopra, 1976) and in Timmiella anomala (Chopra & Rekhi, 1979). Similar observations were made during gemmae and bud initiation. According to Nebel and Naylor (1968a) processes leading to protonemal growth in Physcomitrium turbinatum are metabolically quite separate from those leading to bud initiation. Spiess et al. (1971) reported that in Pylaisiella selwynii age of the protonema rather than its size is critical factor for bud initiation. Similar observations were made in Bryum argenteum (Bhatla, 1983). In contrast, Lal (1961) observed bud formation on chloronemal filaments in Leucodon sciuroides. Rashid (1970) reported bud-free protonema of Anoectangium thomsonii even at caulonema stage. Nair and Raghavan (1976) reported bud formation on both chloronemal and caulonemal filaments of Microdus miquelianus on a cytokinin-supplemented medium. Cytokinin supplemented medium has been reported to support bud formation directly on germinating spores in Entodon myurus (Sood & Chopra, 1973). Yoshida and Yamamoto (1982) observed that in Physcomitrium sphaericum even caulonema cells which are at position less than 15 cells away from a spore do not form buds while chloronemal cells present near the base of a lateral filaments can form buds.
FACTORS AFFECTING BUD FORMATION

Light

Importance of light in bud formation was realized by Klebs (1893) and Goebel (1896). These bryologists observed that under low light intensity, protonema of *Funaria hygrometrica* do not form buds. Transfer of culture in dark resulted in dedifferentiation of buds to chloronema (Servettaz, 1913; Ubisch, 1913). Even addition of sugar into the medium was not effective in inducing buds (Robbins, 1918). Pringsheim and Pringsheim (1935) observed that in *F. hygrometrica* addition of various carbon and nitrogen sources failed to induce buds in dark. These observations were followed by a few reports of bud formation in the absence of light (Fries, 1945; Keil, 1949; Belkengren, 1962) but these reports were not found dependable due to defective methodology. Naef and Simon (1980) reported that initial exposure of cultures to light for some period leads to bud formation even in dark. Mitra and Allsopp (1959a) suggested that light is required for synthesis of a specific formative substance required for bud initiation in *Pohlia nutans*. In *F. hygrometrica*, addition of sugar advanced bud formation under suboptimal conditions of light (Bopp & Brandes, 1964).

Protonema showed poor ramification under low light intensity and higher light intensity was found to be necessary for bud initiation in *Anisothecium molliculum* (Kumra & Chopra, 1985), *Philonotis lancifolia* (Babbar, 1985), *Pohlia elongata*, *Anisothecium spirale* (Vashistha & Chopra, 1987), *Garckea phascoides* (Sarla, 1987), *Microdus brasiliensis* (Mehta, 1988) and *Timmiella anomala* (Kapur, 1990). Nebel & Naylor (1968 a,b) observed that in *Physcomitrium turbinatum* bud formation is dependent upon a threshold level of some morphogenetic substance which is synthesized under cumulative light dose and is held in store over dark periods. Kinetin was found to replace requirement of light for bud initiation in *Ceratodon purpureus* (Szweykowska, 1963) and *Microdus miquelianus* (Nair & Raghavan, 1976). Chopra and Gupta (1967) inferred two-fold role of light: (a) the non-photosynthetic, which initiates buds and (b) the photosynthetic, which supports the growth of the bud primordial. They demonstrated that kinetin replaces the non-photosynthetic role.
Light quality is also important in bud initiation. Red light stimulates protonemal growth and bud formation in Funaria hygrometrica (Pringsheim & Pringsheim, 1935) and Pohlia nutans (Mitra et al., 1959c). Jahn (1964) and Klein (1967) found blue light inhibitory for bud formation. Mitra et al. (1965) found that certain critical balance between red and blue light is necessary for development of buds into leafy shoots. Red light followed by blue light is stimulatory, whereas blue light followed by red light is inhibitory in Physcomitrium turbinatum. Cove et al. (1978) noticed enhancement of bud formation in Physcomitrella patens by a brief exposure to red light and its reversal by far-red light demonstrating phytochrome involvement in this process. Berthier et al. (1976), Simon and Naef (1981) demonstrated that red/far-red dependence of bud formation is quite separate from the photosynthetic requirement.

**Hydration of the Medium**

In liquid cultures of Polytrichum juniperinum, Bastit (1891) observed that water affected the height and direction of growth of plants as well as arrangement, size, shape and color of leaves. Gurlitt (1918) and Brown (1919) noticed that in liquid culture, buds and leafy plants were promoted only on the walls of the culture tubes. Meyer (1940) observed decrease in bud number in liquid cultures compared to semi-solid nutrient medium. Different species required different degree of hydration for optimum bud formation. Kumra (1981) reported maximum number of buds in Barbula gregaria in liquid medium and with decrease in hydration the number of buds decreased, but in Bryum coronatum maximum buds were produced at 1.6% agar containing media. In many mosses optimum response has been observed at 0.8% agar containing media: Anisothecium molliculum (Kumra & Chopra, 1985), Garckeia phasoides (Sarla, 1987), Pohlia elongata, Anoectangium spirale (Vashistha & Chopra, 1987) and Microdus brasiliensis (Mehta, 1988). Kapur (1990) reported maximum number of buds in Timmiella anomala in liquid medium.

**Sugar**

Keil (1949) reported bud initiation on the protonema of Splachnum in dark when medium was supplemented with 2% sucrose. Mitra and Allsopp (1959a) observed that in Pohlia nutans, increase in sugar level up to 2% enhanced bud
formation but higher levels of sugars and mannitol inhibited bud formation. According to them, there is a pre-requisite of a certain minimal sugar concentration in the protonema for bud initiation. Bopp and Brandes (1964) opined that in *Funaria hygrometrica* sugars help in attainment of certain critical size necessary for bud initiation. Hatanaka-Ernst (1966) raised a mutant of *F. hygrometrica* which formed buds only when medium is supplemented with 2% glucose. On the basis of their study on *Physcomitrium coorgense* Menon and Lal (1974) postulated that sucrose play a role in determination of an apical cell with two cutting faces (sporophytic) or three cutting faces (gametophytic). Rekhi (1978) reported that addition of carbohydrates in *Timmiella anomala* culture did not substitute the non-photosynthetic effects of light. In mosses like *Barbula gregaria, Bryum coronatum* (Kumra, 1981), *Anisothecium molliculum* (Kumra & Chopra, 1985), *Philonotis lancifolia* (Babbar, 1985), *Garckea phasoides* (Sarla, 1987), *Pohlia elongata, Anisotheccium spirale* (Vashistha & Chopra, 1987) and *Microdus brasiliensis* (Mehta & Chopra, 1991), 1% sucrose is found to be optimum for bud formation. Smirnoff (1992) reported that in several moss species drying induced changes in percentage of reducing sugars.

**Auxins**

Hurel-Py (1948) reported that in *Funaria hygrometrica* IAA concentration higher than 0.1mg/l inhibited bud formation, while lower concentration (0.01mg/l) stimulated bud formation. At higher concentration, auxin causes dedifferentiation of buds into protonema (Bopp, 1953; Gorton & Eakin, 1957). Kofler (1951) observed formation of leafless gametophores with numerous rhizoids near the base in response to higher concentration of 2,4-D in *F. hygrometrica*. Mitra and Allsopp, (1959a, b) observed that auxins like NAA, IAA and indoleacetonitrile inhibit bud formation in *Pohlia nutans*. In *Ceratodon purpureus*, auxins inhibited shoot development (Szweykowska, 1966). Hatanaka-Ernst (1966) working on an X-Ray mutant of *Funaria hygrometrica* which had lost its capacity to synthesize auxin, observed that buds were produced only when medium was supplemented with IAA, while the wild type produced buds on basal medium. Exogenous application of auxins induced buds in *Ceratodon purpureus* (Szweykowska, 1962). *Dicranella coarctata* (Kumra, 1981), *Anoectangium strachyanum* (Vashistha, 1986) and *Bryum atrovirens* (Chopra &
Vashistha, 1990). Sarla and Chopra (1987) observed that all the auxins (IAA, 2,4-D, NAA) except NOA failed to induce buds in *Bryum pallescens*, but all these auxins promoted bud formation in *Garckeia phasoides* (Chopra & Sarla, 1986). Kapur (1983) reported that in *Timmiella anomala* bud formation was stimulated by IAA as well as NAA at lower levels, and inhibited by 2,4-D and NOA. In *Anisothecium molliculum*, all the tested auxins promoted bud formation at lower concentrations (Kumra & Chopra, 1985).

Lehnert and Bopp (1983) opined that there exists a sequential interaction between auxin and cytokinin in regulating caulonema differentiation and subsequent formation of buds. Kapoor and Bhatla (1993) compared tryptophan synthase activity in 10-days old protonema compared to 13-day old protonema in wild type *Funaria hygrometrica* and its auxin-sensitive mutants (NAR-2, 87.13). Mutants were found to have suppressed IAA biosynthesizing capacity as compared to wild type. So the mutants, due to low availability of endogenous IAA, remained in chloronema stage. Johri and co-workers have characterized certain auxin-binding and GTP-binding proteins from the moss *Funaria hygrometrica* using immunological and affinity labeling methods (Johri & Panigrahi 1998, Panigrahi & Johri, 1998). Bhatla *et al.* (1998) observed auxin modulated changes in cytosolic (Ca$^{2+}$) and activated calmodulin in the protonema of *F. hygrometrica*.

Rohwers and Bopp (1985) reported that in moss *Funaria hygrometrica* both ACC content and ethylene formation are promoted by exogenous IAA thus suggesting ethylene-mediated auxin responses.

**Cytokinins**

Polytrichum juniperinum (Nehlsen, 1979), Timmiella anomala (Chopra & Rekhi, 1979), Barbula gregaria (Kumra 1981), Anisotrichium molliculum (Dua, 1983), A. spirale (Vashistha, 1986), Garckea phascoides (Chopra & Sarla, 1986) and Philonotis lancifolia (Chopra & Dhingra-Babbar, 1988). In case of Tortella caespitosa (Gorton & Eakin, 1957; Gorton et al., 1957) and Amblystegium riparium (Kato et al., 1980), kinetin application failed to alter the time of bud induction, but increased the number of buds. In case of T. caespitosa, the inhibitory effect of IAA is annulled by kinetin application. Abel et al. (1989) reported that a mutant of moss Physcomitrella patens, which was defective in bud formation and in chloroplast division, produced buds on exogenous application of cytokinin.

Cytokinins induce buds in the species which normally do not form buds under ordinary culture conditions like Anoectangium thomsonii (Chopra & Rashid, 1969a). Microdus miquelianus (Nair & Raghavan, 1976), Bryum pallescens (Sarla & Chopra, 1985) and Anoectangium stracheyanum (Vashistha, 1986). Cytokinins bring about bud initiation under non-inductive conditions like suboptimal light level in Pohlia nutans (Mitra et al., 1959b, 1962, 1965), under blue light (Mitra et al., 1965; Jahn, 1964) and even under dark in Ceratodon purpureus (Szweykowska, 1963).

Kinetin application induced buds even on isolated 5-celled long caulonemal filaments of Funaria hygrometrica (Bopp & Diekmann, 1967). Sood and Chopra (1973) observed bud formation soon after spore germination in Entodon myurus on cytokinin-supplemented medium. Bopp (1990) noticed that bud formation in nature is induced at least in part by exogenous cytokinins secreted by rest of protonemal mass.

Bopp (1990) observed that buds induced by the application of cytokinin do not develop into leafy gametophores but become necrotic. Hadeler et al. (1995) reported that addition of cytokinin leads to premature as well as enhanced bud formation in *Physcomitrella*. Reutter et al. (1998) used ipt gene (isopentenyl pyrophosphate gene) which produces numerous active and inactive cytokinins of *Agrobacterium tumefaciens* to transform two developmental *Physcomitrella* mutants. One mutant is defective in budding (P24) and the other is a double mutant defective in plastid division (pdi) and in gametophore development (gad) resulting in malformed buds which could not develop into leafy gametophores. It was noticed that ipt expression resulted not only in a substantial increase in the number of buds per protonema, but also in premature budding. Further, the buds induced by the expression of the bacterial ipt gene were able to develop into leafy gametophores.

Rashid (1968) reported that in *Anoectangium thomsonii*, cytokinins 2iP, kinetin and BAP were effective in decreasing order. Szweykowska et al. (1969, 1971, 1972), Valadon and Mummery (1971) reported effectiveness of FUP, kinetin, BAP and 2iP in an increasing order in *F. hygrometrica*. In *Entodon myurus* 2iP was found to be most effective and tricanthine the least effective in bud induction (Sood, 1972). Cytokinin ribosides are not so effective in bud induction in *F. hygrometrica* (Whittaker & Kende, 1974; Gardner et al., 1978). Spiess (1975) noticed ineffectiveness of cis-zeatin in contrast to trans-zeatin in bud induction in *Pylaisiella selwynii*. BAP was most effective in bud induction in *Bryum pallescens*, *B. atrovirens*, *Anoectangium stracheyanum* and *A. bicolor* (Sarla & Chopra, 1985; Vashishtha, 1986; Saini, 1994). Vashistha (1986) reported 2iP to be most effective in *Anisothecium spirale* and kinetin was found to be most effective in *Pohlia elongata*. Wang et al. (1981 a, b), Perry and Cove (1986), Atzorn and Bopp (1992) reported isopentenyl adenine type cytokinins as major cytokinins in mosses in contrast to zeatin which is present only in minor quantities.

Attempts have been made to understand the mechanism of cytokinin action and it has been suggested that kinetin causes synthesis of some specific proteins in response to kinetin treatment (Bopp, 1961; Szweykowska & Handszu, 1965; Szweykowska & Schneider, 1967). Brandes and Bopp (1965), Brandes (1967 a, b)
reported that kinetin activates synthesis of RNA required for bud formation. Brandes and Kende (1968), opined that cytokinins cause bud formation on specific cells of proper physiological age. Removal of cytokinin during early stages causes reversal of bud to protonema stage. Thus hormone presence is required for critical period of time till differentiation is stabilized. Brandes and Kende (1968) also noticed that washing of cytokinin from the stimulated protonema during the first 72 hours of bud formation leads to reversion of buds.

Regarding the internal changes accompanying bud induction, Schneider et al. (1969) noticed 15 times increase in total RNA content of bud primordial in response to kinetin treatment as compared to protonema cells. Spychala et al. (1975, 1976) reported that during early phase of bud formation there is a decrease in RNAase activity by an inhibition of de novo synthesis of the enzyme followed by a subsequent increase. According to Bopp (1974 a,b) differential gene activity causes a rapid increase in mRNA in specific caulonema cells. He opined that two mechanisms are important for bud formation: a mechanism of cytokinin uptake and another concerned with bud initiation. Bopp et al. (1978) and Erichsen et al. (1977) postulated that certain caulonema specific proteins (CSPs) are produced in caulonema cells in response to cytokinins. Erichsen et al. (1978) stated that cytokinin uptake is independent of the cell type. Later on, Bopp (1980) opined that CSPs are not solely responsible for the target character of cells. However, Reski and Abel (1985) failed to notice any caulonema specific proteins. Neuenschwander et al. (1994) suggested that in Physcomitrella cytokinin induces developmentally synthesis of an extracellular protein.

Production of buds by protonema is a qualitative response to cytokinin exposure. Bud development, as against side branch formation, has been shown to involve qualitative changes in nuclear migration and division, in orientation of cell plates and even a change from tip growth to homogenous growth of the cell walls (Saunders & Hepler, 1982; Steer, 1985). Many studies have suggested role of calcium in cytokinin induced bud induction process (Saunders & Hepler, 1981, 1982, 1983; Saunders, 1986; Conrad & Hepler, 1988). Conrad et al. (1986) reported an increase in
intracellular membrane associated Ca$^{2+}$ and change in polarity of the organelles during hormone activated budding.

Hahn and Bopp (1968) and Valadon and Mummery (1971) showed that bud number is a log linear response from $10^{-8}$ to $10^{-6}$M cytokinin. Bopp (1990) proposed a quantitative response to cytokinin concentration based on the number of cells initially triggered by the hormone. According to him there are number of classes of target cells each increasingly insensitive to cytokinin. Thus, target cells are differentially sensitive to cytokinin. At very low concentration only the most sensitive cells would be triggered; at higher concentration of hormone additional classes of less sensitive cells could be triggered to undergo initial calcium cascade and develop into additional buds.

Kakimoto (1996) implicated a histidine kinase homologue in cytokinin signal transduction. Christianson (1998) proposed that in *F. hygrometrica* initial perception of cytokinin does not control bud number but exogenous cytokinin concentration exerts its quantitative effect on bud number long after initial perception, during time when bud initials become stably committed buds. Many researchers reported that auxin and cytokinin interaction affects process of bud initiation. IAA and Kinetin were reported to exhibit antagonistic effects in *Tortella caespitosa* (Gorton & Eakin, 1957), *Splachnum ampullaceum* (Maltzahn, 1959), *Pohlia nutans* (Mitra et al., 1962) and *Entodon myurus* (Sood & Chopra, 1973). Cytokinin and IAA were noticed to act synergistically in terms of time required for bud initiation, their number and improving morphology of shoot in several mosses like *F. hygrometrica*. *Ceratodon purpureus* (Szweykowska, 1962, Szweykowska & Maćkowiack, 1962, Szweykowska et al., 1969), and *Anoectangium thomsonii* (Chopra & Rashid, 1969a). However, Spiess et al. (1972, 1973) failed to observe any interaction of IAA and kinetin in *Pylaisiella selwynii*. Johri (1974), Johri and Desai (1973) reported that IAA induces caulonema formation which is a pre-requisite for cytokinin action in *F. hygrometrica*. Sood and Hackenberg (1979) reported that on intact protonema in *F. hygrometrica*, IAA, 2,4-D, and tryptophan individually promote bud formation. In contrast, in isolated filaments bud formation was stimulated only by combination of these with cytokinins and amino acid. Vashistha (1986) noticed in *Anoectangium stracheyanum*
and *Bryum atrovirens* combination of BAP and IAA does not significantly affect bud number and the time of their initiation and reduced bud number as noticed in *Garckeia phascooides* (Chopra & Sarla, 1986), and *Philonotis lancifolia* (Chopra & Dhingra-Babbar, 1988).

Attempts have been made to understand the mechanism of auxin and cytokinin interaction. Maltzahn (1959) demonstrated auxin and cytokinin interaction in bud formation in *Splachnum ampullaceum*. Ashton *et al.* (1979a, b) demonstrated that in *Physcomitrella patens* both endogenous auxin and cytokinin play interdependent role in several steps of gametophytic development. In *Dicranella coarctata* normal gametophores are formed only with an interaction of Kinetin and IAA (Kumra, 1981). Auxin transforms cells into target cells for cytokinin and the interaction of the two hormones is a sequential one (Lehnert & Bopp, 1983; Bopp & Atzorn, 1992). Reutter *et al.* (1998) observed that in *Physcomitrella patens* the bud, pdi and gad mutations are not linked to reduced levels of auxin and cytokinin but may affect hormone signal transduction.

**Gibberellins**


### Abscisic Acid

Valadon and Mummery (1971) reported that abscisic acid reduced the number of buds produced in response to kinetin in F. hygrometrica. In Bryum coronatum high concentration of ABA was found to delay bud initiation (Rawat, 1976). ABA stimulated bud initiation in Barbula gregaria. ABA induced buds in Dicranella coarctata which remains bud-free on basal medium (Kumra, 1981). In Timmiella anomala ABA alone and also in combination with kinetin reduced the bud number (Chopra & Kapur, 1989). ABA was found ineffective for bud induction in Anoectangium stracheyanum and Bryum atrovirens (Vashistha, 1986). Chopra and Mehta (1991) reported that in Microdus brasiliensis, combination of ABA and kinetin reduced the number of buds. However, this combination had synergistic effect in Hymenostylium recurvirostrum and in Campylopus richardi this combination induced buds.

Werner and co workers reported that exogenously applied ABA initiates the synthesis of new protein and influences proteins phosphorylation in Funaria hygrometrica (Werner et al. 1991; Werner & Bopp, 1993; Bopp & Werner, 1993). Atzorn et al. (1989a) observed that in the auxin deficient mutant 87.25, ABA not only cause the redifferentiation of protonema into brachycytes but also increases the number of tmema cells.

### Vitamins

Spiess et al. (1973) suggested regulatory role of vitamin B₁₂ in the control of gametophore initiation and development. He reported that in Pylaisiella selwynii vitamin B₁₂ had marked stimulatory effect on bud initiation, but failed to observe any synergistic effect of vitamin B₁₂ and cytokinin combination. Vitamin B₁ and B₁₂ were reported to increase bud number in Barbula gregaria; induced buds in Dicranella coarctata, but were ineffective in Racomitrium lanuginosum (Kumra 1981). In Timmiella anomala vitamins B₁, B₂ and B₆ increased bud number as well as advanced
their initiation (Chopra & Kapur, 1989). Rahbar (1981) observed enhanced bud number in response to vitamin B$_{12}$ in *Bartramidula bartramioides*. Vashistha (1986) reported ineffectiveness of vitamin B$_1$ and B$_6$ in inducing buds in *Anoectangium stracheyanum* and *Bryum atrovirens*. However, B$_{12}$ induced buds in *B. atrovirens*.

**Chelating agents**


**Antiauxins**

Sood and Hackenberg (1979) observed that in *Funaria hygrometrica* PCIB completely counteracts the effect of IAA on bud formation. Vashistha (1986) reported stimulation of bud formation by MH in *Anisothecium spirale*, and by TIBA in *Pohlia elongata* but MH and TIBA failed to induce buds in *Anoectangium stracheyanum*. Sarla (1987) observed increase in bud number in *Garckea phascoides* in response to MH. Sarla and Chopra (1987) reported that in *Bryum pallescens* MH induced buds, whereas TIBA failed to do so. PCIB and its combination with kinetin inhibited bud formation in *Philonotis lancifolia* and *Trematodon brevicalyx* (Chopra & Dhingra-Babbar, 1988; Dhingra-Babbar & Chopra, 1990). Chopra and Mehta (1992) observed that in *Microdus brasiliensis* MH and TIBA stimulated bud formation while in *Campylopus richardii* only MH induced buds.
Heavy Metals

Mosses have a long and extensive history of use in the study of heavy metal toxicity on plants and on natural ecosystems (Tyler, 1990). Bioassays on moss spore germination have been used in the assessment of heavy metal ion toxicity (Coombes & Lepp, 1974; Lepp & Roberts, 1977; Petersen & Francis 1980).

Bryophytes have the capacity to accumulate atmospheric pollutants and after some investigations in Sweden bryophytes have been used for monitoring of heavy metals (Rühling & Tyler, 1968; Thomas, 1986; Tyler 1990; Wegener et al., 1992).

Zinc: Biebl (1947) reported that high concentration of zinc sulphate caused damage to the leaf margin and mid rib cells of young leaves in *Plagiomnium rostratum* before the spread of necrosis to whole of the gametophyte. Url (1956) reported high tolerance to zinc due to immobilization of metals in a surface coating surrounding the tissues in two species of *Mielichhoferia, M. elongata* and *M. nitida*.

Pickering and Puia (1969) studied the mechanism of zinc uptake by *Fontinalis antipyretica* and observed that three successive stages are involved in this process. The effect of distance from source (industries or smelters) on the concentration of zinc in moss tissues has been reported in *Eurhynchium praelongum* (Burkitt et al., 1972 cited in Rao, 1982), *Hypnum cupressiforme* and *Hypnum splendens* (Rühling & Tyler, 1969, 1970; Tyler, 1970; Le Blanc et al., 1974). Coombes and Lepp (1974) observed that high levels of zinc inhibited bud formation in *Funaria hygrometrica* and resulted in formation of numerous rounded brood cells containing reddish granular inclusions. These workers also reported toxic effects of zinc in gemmalings of *Marchantia polymorpha*. Czarnowska and Rejment-Grochowska (1974) observed 45 ppm of zinc level in gametophyte of *Atrichum undulatum* and 75 ppm in sporophyte. Urban and rural populations of two feather mosses *Pleurozium schreberi* and *Hylocomium splendens* were studied by Barclay-Estrup and Rinnie (1978) for their ability to accumulate zinc. They found that *P. schreberi* accumulated larger amount of zinc and zinc concentration was higher in urban areas as compared to rural areas. Burton and Peterson (1979a) made investigations on zinc localization in mine stream bryophytes and in laboratory cultured *Fontinalis antipyretica*. Burton
and Peterson (1979b) reported that in *Scapania undulata* 80-90% zinc was accumulated in the cell wall.

Higher levels of zinc had inhibitory effect on metabolic activities (Haseloff, 1979; Haseloff & Winkler, 1980). Empain (1976) observed that *Fontinalis antipyretica* and *Cinclidotus nigricans* from Belgian and French rivers endured 5000 mg/kg of zinc in their tissues. Species of *Fontinalis* and *Rhyncostegium* accumulated 8000 mg/kg of zinc from a zinc polluted British river (Say *et al*., 1981). Bengtsson *et al.* (1982) observed that in *Hylocomium splendens* branching frequency and chlorophyll content were not influenced by zinc concentration of less than 8-10 times the regional background level in study from a secondary brass smelter. Wehr *et al.* (1983) found that *Fontinalis antipyretica* contains lower concentration of zinc in comparison to *Rhyncostegium ripariodes*. Beckett and Brown (1983) on the basis of their experiments on transfer of a pulse of zinc ions from *Rhytidiadelphus squarosus* cell wall to cell interior concluded that binding of heavy metal to the cell wall does not ensure protection of cell.

Folkeson (1979, 1983, 1984), Folkeson and Andersson-Bringmark (1988) reported that *Pohlia nutans* is most tolerant while quantitatively abundant feather mosses were comparatively sensitive to zinc. Effects of zinc were investigated on spore germination and protonemal growth in *Polytrichum commune* (Francis & Peterson 1989) and protonemal development and bud formation in *Timmiella anomala* (Kapur & Chopra, 1989). In *T. anomala* higher concentration of zinc caused production of round cells with reddish tinge.

Baker (1992) reported inhibition of growth of shoots of two *Sphagnum* species at higher conc. of ZnCl₂ *in vivo* as well under *in vitro* conditions. Gupta and Chopra (1995) while studying growth in *Riccia discolor* observed slow growth, decreased branching reduction in photosynthetic zone in response to increase in zinc concentration. Higher concentration of zinc also did not support archegonial formation. Sidhu and Brown (1996) noticed that in non-extending (moribund) shoots of *Rhytidiadelphus squarosus* continued to accumulate zinc throughout the growth period. Bruns *et al.* (1997) studied correlation between zinc accumulation in *Fontinalis antipyretica* and degree of its pollution in the water of river Elbe. Higher
zinc concentration caused induction of thiol containing peptides such as phytochelatins and glutathione.

**Copper:** Url (1956) reported that *Mielichhoferia elongata* and *M. nitida* are tolerant to very high concentration of copper due to immobilization of the metal in surface coating surrounding the tissue. These species also indicated a real plasmatic tolerance. Persson (1956) found that the moss *Crumia latifolia* grew on substratum containing even 320 ppm copper. Shacklette (1961) observed that in Alaska the bryophytes *Calypogeia muelleriana*, *Cephalozia bicuspidata*, *Gymnocolea denticulata*, *Nardia scalaris*, *Oligotrichum* spp., *Pleuroclada albescens* and *Pohlia nutans* are relieved from competition with vascular plants due to their tolerance to copper. *Pohlia nutans* was reported to contain 87000-35000 mg/kg copper from a natural copper swamp in Canada (Dykeman & De Souza; 1966). *Mielichhoferia macrocarpa* contained higher amounts of copper compared to average amounts present in lithosol and volcanic rocks (Shacklette, 1966). Rühling and Tyler (1968) reported good correlation between annual rainfall and the concentration of copper in epigeic mosses of Sweden. According to Hartmann (1969), *Mielichhoferia nitida* prefers to grow on strongly aberrant cupriferous substrates. *Hypnum cupressiforme* and *Hylocomium splendens* showed an increase in copper content with decreasing distance from an industrialized area and increased precipitation (Rühling & Tyler, 1969). Rühling and Tyler (1970) opined that copper uptake was not exclusively by ion exchange because only two third of copper content was exchanged by repeated leaching with magnesium chloride. Coombes and Lepp (1974) demonstrated toxic effects of copper in gemmalings of *Marchantia polymorpha* and in germinating spores of *Funaria hygrometrica*. At 4 ppm of copper protonemal structure was disorganized and many so called “capsule cells” were observed. Copper level above 8 ppm caused cessation of protonemal growth and inhibition of spore germination in *F. hygrometrica*. Pilegaard *et al.* (1979) while studying atmospheric background deposition of heavy metals in Denmark found that the concentration of copper was highest in *Hypnum cupressiforme*. Copper also caused inhibition of rhizoids initiation and their growth. *Hylocomium splendens*, *Pleurozium schreberi*, *Ptilium crista-castrensis* and *Plagiothecium roescanum* were reported to be sensitive to copper pollution, whereas *Dicranum varia* and *Brachythecium salebrosum* were quite
tolerant (Le Blanc et al., 1974). Czarnowska and Rejment-Grochowska (1974) noticed that in *Atrichum undulatum* copper concentrations were 4.5ppm and 7.4ppm in gametophytic and sporophytic tissues, respectively. Aquatic mosses *Fontinalis antipyretica* and *Cinclidotus nigricans* were reported to contain exceeding high levels of copper (1360-1900 mg/kg) in their tissues (Empain, 1976). Simola (1977b) observed that under high concentration of copper mitochondria had clear cristae. Chloroplast developed large starch grains and plastoglobules, whereas sphaerosomes were small. Copper concentration less than 8-10 times the regional background level did not influence branching frequency of chlorophyll content in *Hylocomium splendens* (Bengtsson et al., 1982). *Pohlia nutans* was observed to grow luxuriantly on copper-rich substratum near a brass smelter (Folkeson, 1983), whereas higher levels of copper was reported to inhibit growth in *Polytrichum* (Klein & Bliss, 1984). Wehr et al. (1983) found *Rhyncostegium ripariodes* to be a good accumulator of copper in comparison to *Fontinalis antipyretica*. Scanning and transmission electron microscopy investigations done by Satake et al. (1988) on *Scopelophila catracta* showed that cell wall was an important site of copper accumulation as compared to other cell components. They also noticed high concentration of copper (1-3%) in the shoots although copper concentration in rainwater was lesser than this. In a study of polluted peatland, in Ontario (Canada), Gignac and co-workers reported *Sphagnum* was absent from peatland where copper levels in water samples rose above 2 µM (Gignac et al., 2000). Baker (1992) observed that in two *Sphagnum* species, inhibition of shoot growth in response to copper chloride occurred at concentration which were about nine times lower than that caused by zinc chloride. He also observed that axenically cultured shoots of *Sphagnum* were killed when supplied with more than 4µM copper chloride.

Sidhu and Brown (1996) in their experiment of a novel technique studying the effects of heavy metals on bryophyte growth, observed that using both methods of exposure of shoot to heavy metal solution (i.e. via cut bases or by pulse immersion) Pb and Cu showed less mobility along the shoot length than Zn and Ni.

Folkeson (1983) while studying deterioration of the moss vegetation in a forest polluted by copper observed that *Hylocomium splendens* is one of the most
sensitive mosses. Yule and Lloyd (1984) found high copper concentration in the moss *Hypnum cupressiforme* in Armadale, Central Scotland. The pattern revealed an increasing concentration from the periphery of the town towards the vicinity of Armadale steel foundary.

Rinne and Barclay-Estrup (1980) found increase in concentration of copper in the feather moss *Pleurozium schreberi* with increasing tree cover and decrease with increasing distance from source of pollution. Czarnowska and Gworek (1992) noted copper concentration of 4-8 mg/kg dry weight in moss *Pleurozium schreberi*. Low *et al.* (1993) observed that dye coating of moss *Calymperes delessertii* improved the sorption capacity of the moss for copper and other heavy metal cations.

Gignac (1987) studied accumulation of copper in the partially humidified peat surrounding the living *Sphagnum* and it was significantly higher than the concentration retained in moss plants, particularly on sites having high metal loading. There existed a significant relationship between metal accumulation in the capitula of *Sphagnum russowii*, *S. magellanicum* and *S. riparium* and partially humidified peat below the living moss.

Absolute uptake efficiency of *Hylocomium splendens* and *Pleurozium schreberi* for copper varied between 0 and 92%. This indicates that metal uptake depends very strongly on microenvironmental conditions (Coburnis & Valiulis, 1999).

**Lead:** Rühling and Tyler (1969, 1970) and Tyler (1972) reported an increase in lead content in *Hypnum cupressiforme* and *Hylocomium splendens* with increasing precipitation and decreasing distance from industrialized area. Brown and Bates (1972) studied lead uptake in two populations of *Grimmia donniana* and there was found almost no difference in their uptake capacities. According to them uptake was a passive process and lead was ionically bound to cell wall, thus preventing its penetration into cytoplasm.

Lee and Tallis (1972) studied the effect of lead pollution from a factory manufacturing antiknock compounds. Lead contents in *Ceratodon purpureus*, *Eurhynchium praelongum*, *Plagiomnium cuspidatum* and *Calliergon cuspidatum*...
growing at variable distance from the factory declined rapidly in the first 400 meters and then slowly in the next 400 meters distance; but at 800 meters, lead content in the mosses was still two to three times higher than that in the species collected from rural areas remote from factory. Burkitt et al. (1972 cited in Rao, 1982) noticed higher concentration of lead in bryophytes growing near the smelter and decrease in lead concentration with increasing distance from smelters. Skaar et al. (1973) noticed lead accumulation in the nuclear membrane of leaf cells of *Rhytidiadelphus squarrosus*. This was suggested to be mechanism restricting cytoplasmic concentration of diffusible lead below a level which could be toxic to lead sensitive cell organelles (like mitochondria) and cell functions. Electron microscopic studies of experimental and environmental lead poisoning of leaf cell of moss *Rhytidiadelphus squarrosus*, showed lead accumulation in cell wall and also in cytoplasm. In leaves of mosses exposed to environmental pollution lead was bound within nucleus as electron dense nuclear inclusion (Gullóag et al., 1974). According to Lee and Tallis (1973) peat accumulated lead up to 600 ppm in polluted areas of England. Mclean and Jones (1975) reported lead tolerant aquatic mosses which do not show sign of damage. Krupsinska (1976) found 0.0003 mM concentration of tetraethyl lead inhibitory to protonemal growth of *Funaria hygrometrica*. High level of (180 mg/kg) lead was recorded in *Sphagnum* growing in north-eastern United States was found to be good lead accumulator (Groet, 1976). Simola (1977a, b) studied the growth of two species of *Sphagnum*, *S. fimbriatum* and *S. nemorum* in response to different lead concentrations. *S. nemorum* was found to be very sensitive to lead. Barclay-Estrup and Rinnie (1978) reported presence of lead within the cells of *Grimmia donniana*. Thomas (1979) analyzed lead contents in *Hypnum cupressiforme* from 37 sites in middle Europe. Lead content was maximum ranging from 16-267 mg/kg from sites near heavy metal industries. Brown and Buck (1985) found lead in an extracellular exchangeable form in thoroughly washed materials of *Grimmia donniana* and *Calliergonella cuspidata*. Onianawa et al. (1986) studied sorption and retention of lead in 3 species of mosses in Nigeria. The mosses *Rhacopilosis irinitensis*, *Stereophyllum virens* and *Thuidium gratum* have very high capacity to absorb lead from single ion and mixed ion solutions. During sorption of heavy metal ions calcium and magnesium were more readily released from the moss. Low et al. (1985) found
that the moss *Calymperes delessertii* is a good indicator for monitoring atmospheric lead. Sharma and Chopra (1987) studied the effect of lead nitrate and lead acetate individually and in combination on protonemal growth, time taken for bud initiation, bud number, shoot length and chlorophyll contents in *Semibarbula orientalis*. Lead nitrate was found to be more toxic and increased concentration resulted in increased toxicity. Kapur and Chopra (1989) also reported inhibitory effect of lead nitrate on *Timmiella anomala*. High concentration of lead was recorded in tissues of *Sphagnum subsecundum*, *Drepanocladius exannulatus* and *Fontinalis flaccida* (Farmer, 1988).

Kardash and Demkiv (1991) studied the inhibitory effect of lead nitrate on protonemal growth in *Funaria hygrometrica*. Basile *et al.* (1993, 1994) used X-Ray, SEM, TEM analysis and atomic spectroscopy to determine tissue and cell localization of lead in plants of *F. hygrometrica* with sporophytes at different phases of development. According to them lead gets sequestered especially in gametophyte hydroids and placental transfer cells and does not reach the upper part of the seta or sporogenous tissue, thus protecting the reproductive sites from toxic action of the metal. Basile *et al.* (1995) studied morphogenesis in protonemata of *F. hygrometrica* in response to different lead concentration. Protonemata showed disturbance in polar growth, changed arrangement of chloroplasts, alterations of nucleus and septa position and these effects were dose dependent. Higher doses caused reduction in protonemal system, high vacuolation and even death.

Satake *et al.* (1989) studied lead accumulation in shoots of aquatic liverwort *Scapania undulata* with respect to its concentration, localization and chemical forms in the cells and showed localization of Pb only in the cell wall. Watkinson and Watt (1992) through scanning proton microprobing in leaf cells of *Sphagnum* suggested abundance of lead in lower, older parts of the plants, perhaps because of passive accumulation on the surface of plants.

**Mercury:** Yeaple (1972) observed that level of mercury in terrestrial mosses increases with decreasing distance from pollution centers. According to Huckabee (1973) *Dicranum scoparium* accumulates good quantity of mercury than *Polytrichum commune* and this difference was attributed to their different life forms. Mondano and Smith (1974) recorded 4.34 ppm mercury in *Dicranella heteromalla* at the urban end
of a transect, and only 0.24 ppm in the rural areas. Siegel et al. (1975 cited in Chopra & Kumra, 1988) studied the mercury content of ten mosses from Alaska and Hawai and reported a range between 0.1 and 120 µg/100g with an average of 13 µg. 

Hypnum cupressiforme was found to accumulate mercury between 9 to 15 µg/100g (Wallis, 1976). An average mercury concentration of 123 µg was found in Hylocomium splendens collected from 43 sampling sites in Norway (Steinnes, 1977). According to Simola (1977c) 10⁻² mM mercury could not induce much change in ultrastructure and growth of Sphagnum fimbriatum, but 20 mg/1 mercury proved lethal. Solberg and Selmer-Olsen (1978) determined mercury level of 11 species of mosses. Out of these Tomenthypnum nitens accumulated about 94 µg/100g of mercury while the other ten had an average of 16.6 µg/100g. The process of spore germination in Polytrichum commune was found to be 10 times sensitive to mercury than the fern Onaclea sensibilis (Peterson & Francis, 1980). Complete inhibition of photosynthesis occurred in Pseudoscleropodium purum in response to mercury (Haseloff, 1979). Rinne and Barclay-Estrup (1980) found mercury content of 0.06 ppm dry weight in Pleurozium schreberi and concentration increased with decreasing distance from the source of pollution. Satake and Miyasaka (1984) reported that Jungermannia vulcanicola from an acidic stream accumulated Hg in the cell wall as electron dense particles. Brown and Whitehead (1986) reported that low concentration of mercury severely affect substantial loss of intracellular potassium in the moss Rhytidiadelphus squarrosus. All these alteration were due to membrane damages as a result of rise in mercury level. Mercury induced brood cells with many dense particles in Timmiella anomala (Kapur & Chopra, 1989). Tyler (1990) observed that 0.005 mM of mercury had adverse effects on physiology and 0.1 mM mercury caused complete inhibition of growth and photosynthesis in bryophytes.

Skacel and Pekarek (1992) monitored mercury in three moss species: Hylocomium splendens, Pleurozium schreberi and Rhytidiadelphus squarrosus. A good correlation was found between mercury contents of all samples. Mankovska (1996) studied mercury concentration in forest trees from Slovakia. The mercury contents in the soil from a mercury smelting plant ranged from 9.9 to 1.0 mg/kg and the moss Pleurozium schreberi contained 3.8-9.1 mg/kg showing high accumulation capacity of moss.
**Cadmium:** Tyler and coworkers (Rühling and Tyler, 1969, 1970; Tyler, 1972) reported increase in cadmium contents in *Hylocomium splendens* and *Hypnum cupressiforme* with increasing precipitation and decreasing distance from industrialized area. Similar observations were also made by Burkitt *et al.* (1972 cited in Rao, 1982) and Le Blanc *et al.* (1974). Lepp and Roberts (1977) reported inhibitory effect of cadmium on spore germination in *Funaria hygrometrica*. Cadmium contents of 5.1 mg/kg dry weight was noticed in *Sphagnum* growing in polluted bogs of Sweden (Pakarinen & Tolonen, 1976). Simola (1977, b, c) observed the effect of different concentrations of cadmium on the growth of *Sphagnum* growing in polluted bogs of *S. fimbriatum* and reported it to be a cadmium tolerant species. *Dicranoweissia cirrata* transplanted to various Danish sites, was also reported to accumulate cadmium (Johnsen *et al.*, 1983). Cadmium nitrate had inhibitory effect on the growth of *Pseudoscleropodium purum* (Haseloff, 1979; Haseloff & Winkler, 1980) *Rhacopilopsis trinitensis*, *Stereophyllum virens* and *Thuidium gratum* have very high capacity to absorb cadmium from single ion and mixed ion solutions. (Onianawa *et al.*, 1986). Brown and Buck (1985) reported that the moss *Rhytidiadelphus squarrosus* has a high cadmium affinity, high cadmium uptake rate, and high extracellular cadmium binding capacity. *Hypnum cupressiforme* accumulate cadmium from rain water and air (Thomas, 1986). Wehr *et al.* (1983) found that *Rhyncostegium riparioides* absorb more cadmium in comparison to *Fontinalis antipyretica*. Francis and Peterson (1989) while comparing the effect of cadmium with zinc and copper on spore germination and protonemal growth of *Polytrichum commune* found cadmium to be more toxic than zinc and less toxic than copper. Wells and Brown (1987) observed movement of extracellular cadmium to an intracellular location on exposure of bryophytic tissue to cadmium sulphate solution. *Sphagnum fuscum* was reported as bioindicator of cadmium deposition across Canada (Glooschenko, 1989).

Clement and Werner (1989) studied heavy metal content of cadmium in the moss *Mnium hornum* in beach forest of Germany. It was found that the cadmium content of the moss increased with increasing proximity to the industrial rural area.

Czarnowska and Gworek (1992) observed 0.6-0.9 mg/kg dry weight content in the moss *Pleurozium schreberi*. Skacel and Pekarek (1992) monitored cadmium in
Hylocomium splendens, Pleurozium schreberi and Rhytidiadelphus squarrosus and found that cadmium content in the environmental samples were comparable to reported natural levels. Siebert et al. (1996) studied distribution of cadmium in Fontinalis antipyretica. It was observed that the moss contained higher concentration of cadmium and there was less biological dispersion in older parts of the plants than in young shoot tips.

Bruns et al. (1997) studied cadmium accumulation and its physiological reaction in F. antipyretica. A correlation was found between the cadmium content in the moss and degree of cadmium pollution in water. A positive correlation was also found between phytochelatin levels and cadmium levels in moss samples.

**Nickel:** Tyler and co-workers (Rühling & Tyler 1969, 1970; Tyler, 1972) observed increase in concentration of nickel in Hypnum cupressiforme and Hylocomium splendens with increasing precipitation and decreasing distance from smelting industries. They also found that nickel accumulation in Hylocomium is a passive process. Nickel uptake increased with increasing pH and buffering action may account for significant nickel uptake (Nieboar et al., 1976). Three epiphytic cryptogams, a moss (Hypnum cupressiforme) and two lichens were used for analyzing atmospheric background deposition of nickel. (Pilegaard et al., 1979). The concentration of the plant material reflected the regional atmospheric deposition of the heavy metal. Highest concentration was recorded in Hypnum. Kapur and Chopra (1989) observed significant growth stimulation in Timmiella anomala at low level of nickel (0.5mg/1). At higher concentrations it proved inhibitory. Higher nickel concentration induced thin, sparingly branched, yellowish-green filaments and also reduced the number of buds. Bargagli et al. (1995) showed lowest tip to base ratio of nickel which has a lower degree of mobility in various weathering environments.

Studied done by Wehr et al. (1983) indicate that Rhyncostegium riparioides is a more efficient bioaccumulator of nickel than Fontinalis antipyretica. Rinne and Barclay-Estrup (1980) observed ranges of background metal concentration on Pleurozium schreberi, 50 to 200 km from source of pollution. Nickel concentration in the moss was found to be 2.0-3.4 ppm dry weight. Czarnowska and Gworek (1992) observed 4.8 mg/kg of dry weight of nickel in the moss Pleurozium schreberi.
Gignac (1987) studied ecological tolerance of *Sphagnum* along a pollution gradient near Sudbury, Canada. Accumulation of nickel in the partially humidified peat surrounding the living *Sphagnum* were significantly higher than the concentration retained in the moss plants, particularly on sites having high metal loading. A significant relationship was seen between metal accumulation in the capitula of *Sphagnum russowii*, *S. magellanicum* and *S. riparium* and partially humidified peat below the living moss.

Kakulu (1993) used *Polytrichum commune* to assess nickel pollution at 56 sites in Nigeria. No significant difference was recorded for urban and forest sites. Ho et al. (1996) studied kinetics of competitive heavy metal adsorption by *Sphagnum* moss peat pore diffusion of copper and nickel from both mono and bi-solute system. But presence of contaminant copper ions reduced the binding of nickel.

**Arsenate**: Simola (1977b,c) observed that arsenate concentration lower than 1mM inhibited the growth of *Sphagnum nemoreum*. At this concentration shoots turned yellowish-green, cytoplasm degenerated, chloroplast membranes were damaged and thylakoid stored starch and swelled. This is thought to be due to differentiation of hyaline cells with thickened cell walls which decreased the amount of glucose needed for cellulose synthesis so that more glucose is available for starch synthesis. This detoxification mechanism thus saved the plant at lower arsenic levels but 1mM concentration proved lethal. Kapur and Chopra (1989) observed that in *Timmiella anomala* potassium arsenate reduced protonemal growth and delayed bud initiation. It also adversely affected bud number and gametophores development. Protonemal filaments showed terminal or intercalary spherical cells in presence of even low levels of arsenic.

**Chromium**: Lounamaa (1956) reported 99 µg/g of chromium content in 16 bryophytic species. Shacklette (1965) reported an average of 79 µg/g ash weight of chromium in 38 species of mosses. Tyler and co workers (Rühling & Tyler, 1969, 1970; Tyler, 1972) reported an increase in chromium content in *Hypnum cupressiforme* and *Hylocomium splendens* with increasing precipitation and decreasing distance from smelters. Epiphytic moss *Aerobryopsis longissima* is reported to accumulate 5000 µg/g of chromium from bark of tree and also from the
water dripping from its leaves. It is the highest record for bryophytes (Lee et al., 1977). Moss bags containing moss *Rhytidium triquertus* and *Isothecium myurum* used as collectors of airborne chromium in the industrialized area in Spain (Tarazona-Lafarga et al., 1987). Pilegaard et al. (1979) studied atmospheric background deposition of heavy metals in Denmark by using moss *Hypnum cupressiforme* and two lichens. Concentration of chromium was found to be highest in *Hypnum*.

In a study done by Berg and Steinnes (1997) *Hylocomium splendens* was found to contain higher concentration of chromium as compared to *Pleurozium schreberi*.

**Interaction of heavy metals:** Francis and Peterson (1989) noticed synergistic toxic effect of copper and cadmium as well as cadmium and zinc on spore germination and protonemal growth in *Polytrichum commune*. The combination of copper, cadmium and zinc elicited synergistic response for spore germination and additive to slightly synergistic response for protonemal growth. Kapur and Chopra (1989) observed that in *Timmiella anomala*, zinc and cadmium combination decreased the diameter of protonemal patch and the inhibitory effect of cadmium and zinc on bud number was reduced when the two were added together, but time of bud initiation remained unaffected. Co-addition of cobalt and nickel had different effect on protonemal growth and bud formation. Cobalt reduced the stimulatory effect of nickel on protonemal growth, but it decreased the inhibitory effect of nickel on bud formation.

**Antimicrobial studies**

Many bryophytes have been reported to possess medicinal properties (Watt, 1891; Wren, 1956; Hartwell, 1971). Observations that bryophytes are not attacked by parasitic organisms suggested presence of some antimicrobial compounds in them. Madsen and Pates (1952) and Pates and Madsen (1955) studied antimicrobial activities of eight bryophytes. They reported that *Conocephalum conicum*, *Dumortiera hirsuta*, *Sphagnum portoricense* and *S. strictum* were active against certain microorganisms. Ramaut (1959) showed that extract of *Sphagnum* inhibited growth of bacterium *Sarcinia lutea*. Mc Cleary and Walkington (1966) reported 18 mosses that
showed strong inhibition to gram positive and gram negative organisms because of their antibiotic properties. Gupta and Singh (1971) reported highest antimicrobial activity in petroleum ether extract of *Barbula* sp. and *Timmiella anomala* against gram positive and gram negative bacteria. Banerjee and Sen (1979) reported active antibacterial activity of many bryophytes and concluded that degree of antibiotic activity depends on age of the plant, season of collection and the ecological niche. Extracts from bryophytes have been used to cure dermatitis (Ando & Matsuo, 1984). The inhibitory effect of bryophytes on microorganisms was tested on phytopathogenic bacteria and fungi viz. *Alternaria kikuchiana*, *Aspergillus fumigates*, *Aspergillus niger*, *Candida albicans*, *Microsporum gypseum*, *Pyricularia oryzae*, *Rhizoctonia solani*, *Saccharomyces cerevisiae* (Asakawa, 1994). Basile et al. (1998) tested an acetone extract of *Rhyncostegium riparioides* against 11 bacterial strains. Frahm (2004) reported the ability of mosses to cure fungal and bacterial infections in horses. The extract was active on some gram negative strains. *Atrichum* is used as antibacterial and anti-inflammatory agents in Chinese medicines (Glime, 2007). Sabovljevic et al. (2006) evaluated the antimicrobial activity of *Bryum argenteum* ethanol extracts against four bacterial and fungal species. It proved effective against all bacteria and fungi tested. Zhu et al. (2006) suggests that bryophytes are one of the most significant and promising source of antibiotics and biological active compounds in nature. Bodade et al. (2008) revealed that ethanolic extract of bryophytes showed significant activity against tested bacteria and fungi. Chaudhary and Khanum (2011) reported antimicrobial potential in *Physcomitrium coorgense*. 