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A New Look at Stoic Ethical Precept for Sustainability

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ABSTRACT

This paper seeks to run back over the Stoic Seneca's ethical precept: "live or life according to Nature" once again for the realization or restoration of sustainability. Though ancient Greek Stoicism is no longer existent, yet Stoic Seneca's ethical precept has not been forgotten. Like tradition, the Nature embedded ethical precept of Stoicism should or can be defended, but not in the traditional way, since tradition defended in traditional way implies fundamentalism.

Key Words: Stoicism, Nature, Conservation, Fundamentalism, Precept

1. Introduction

In the age of sustainability revolution, which has started its effective life since the 1970s, various preconditions, precautions, principles and/or policies are being adopted for the conservation of Nature. But, without clear-cut understanding of Nature, and the way of its conservation, how is it possible to conserve Nature? Should we not decide today what nature is and how we should reorganize our way of living in relation to it before taking conservation action? Though ancient Greek Stoicism is no longer existent, yet Stoic Seneca's ethical precept: "live or life according to Nature" has not been forgotten. Like tradition, the Nature embedded ethical precept of Stoicism should or can be defended, but not in the traditional way, since tradition defended in traditional way implies fundamentalism. Today's humanized or socialized Nature and ecology should or can be conserved or restored, but not in the traditional way. Nature, ecology and tradition are equivalent in the sense that these are erroneously treated as pre-given and independent of "humanization or socialization" (Konar, 2013). Their restoration should never deliberately be backed up by fundamentalism. This paper seeks to run back over the Stoic Seneca's ethical precept once again for the realization or restoration of sustainability (Konar & Chakraborty, 2011).

2. Conflicting Perspectives of Nature

Humans are advised by the Stoic ethical precept to "live according to Nature". But, if conflicting perspectives of Nature are existent in the interdisciplinary literature, then Stoic ethical precept must involve conflicting pathways. Then, comes the problem of choice from the multitude of pathways. Now, our discussion will be concentrated on the conflicting perspectives of Nature on which the Stoic ethical precept is based. The perspective of Nature is shaped by social as well as ecological frameworks, since humans are impacted by both the social and ecological factors, given the natural instability indicated by natural catastrophes and natural stability indicated by the equilibrium of various natural life support systems. Further, the perspective of Nature is determined by the following two factors:

(i) The ways of human interaction with the Nature determine the ways of human perception and interpretation of Nature

(ii) The ways of human perception and interpretation of nature determine how humans interact with the Nature.

In this context Milton's (1997) remark is noteworthy. He says that "cultural perspectives thus ……. guide human activity. This activity, in turn, yields experiences and perceptions, which shape people's understanding of the world. The process is not unidirectional, but dialectical".

Moreover, how the perspective of Nature is embedded in the minds of the people should be looked at from two sets of peoples such as:

(i) Peoples who act as analysts, scholars, researchers, authors or scientists (observers)

(ii) Peoples who are studied by the former (observed).

In this connection, the comment of Dawkins (1995) should be recalled: "we are just brought up in a culture that sees the world in a scientific way. They (tribe, who believe that the moon is an old calabash tossed into the sky, hanging only just out of reach above the treetops) are brought up to see the world in another way. Neither way is more true than the other". In a similar vein, Dwyer (1996) has argued that the capacity of a particular society to develop a concept of Nature depends on whether they view their environment as an integrated whole or divide it into familiar and unfamiliar spaces, and this, in turn, depends on how they live in and their environment. He suggests that, in the fully integrated world of the Kubo, there is no sphere sufficiently distinct from the human world to merit the label Nature, while the Siane environment contains unused and familiar spaces, which might be so labeled. Further, Ingold (1996) says that hunter-gatherer communities do not have a concept of Nature because the world can only be Nature for a being that does not belong there. Similarly, Howell (1996) points out that the jungle in its totality as a material and spiritual world is …….cultural space, not natural. They (Chewong of Malay rainforest) move around in it with confidence derived from understanding and knowledge. In this case, the label is applied by the analyst, there is no suggestion that the people themselves would describe their environment in this way.

Various perspectives of Nature are existent in the interdisciplinary literature. However, Beck's (1994) notion of Nature should be prioritized first as follows:

Nature is not nature, but rather a concept, norm, memory, utopia, counter-image. Today more than ever, now that it no longer exists, nature is being rediscovered, pampered. The ecology movement has fallen prey to a naturalistic misapprehension of itself…. 'nature' is a kind of anchor by whose means the ship of civilization, sailing over the open seas, conjures up, cultivates, its contrary: dry land, the harbour, the approaching reef.

Beck's remark cannot cease us from searching for the definitions of Nature. For evidences show that A.D. Lovejoy was capable of counting over sixty different shades of meaning of Nature. The preferred notions of Nature are given below:

One of the clearest statements of the idea of Nature has been depicted in English literature in terms of *Pope's Essay on Man* as follows (Cuddon, 1998):

Vast chain of being! Which from God began,
Natures ethereal, human, angel, man,
Beast, bird, fish, insect, what no eye can see,
No glass can reach; from Infinite to thee,
From thee to nothing. ---On superior pow'rs
Were we to press, inferior might on ours;
Or in the full creation leave a void,
Where, one step broken, the great scale's destroy'd;
From Nature's chain whatever link you strike,
Tenth, or ten thousandth, breaks the chain alike.

The classic work on the notion of Nature is found in A.O. Lovejoy's *The Great chain of being: A study of the history of an idea* (1936).

A considerable emphasis on imitating the law of Nature is seen in pope's *An Essay on Criticism* (1711) [Cuddon, 1998] as follows:

First follow Nature, and your judgement frame
By her just standard, which is still the same;
Unerring NATURE, still divinely bright,
One clear, unchang'd and universal light.

According to Whitehead (1953), "The whole life of Nature is dominated by the existence of periodic events, that is, by the existence of successive events so analogous to each other that, without any straining of language, they may be termed recurrences of the same event,"

Ellen (1996) has identified three distinct senses in which Nature is understood in Western society:

(i) As space which is not human

(ii) As a category of things

(iii) As inner essence, where Nature seen as (ii) and (iii) includes both human and non-human beings.

Escobar (1997) views that Nature can no longer be seen as an essential principle and foundational category, an independent domain of intrinsic value and truth but as the object of constant reinventions, especially by unprecedented forms of technoscience.

In the eyes of Sheldrake (1991), Nature is alive, which oppose mechanistic approach to Nature in which Nature is treated as an inanimate sources of natural resources. Sheldrake (1991) says that such a view is implicitly feminine, for the words Nature and natural have their origins in the mothering process. How Nature is seen through the eyes of the famous poet Hopkins [Dyson, 1995] will be obvious from the following lines of his poem *Brothers*:

> Ah Nature, framed in fault,
> There's comfort then, there's salt;
> Nature, bad, base, and blind,
> Dearly thou canst be kind;
> There dearly then, dearly
> I'll cry thou canst be kind.

Giddens's (1994) conceivability of Nature is as follows:

> The paradox is that nature has been embraced only at the point of its disappearance. We live today in a remoulded nature devoid of nature… nature can not any longer be defended in the natural way…socialized nature is by definition no longer natural. The longing for a return to nature…. is a healthy nostalgia… Nature has come to an end in a parallel way to tradition…Instead of being concerned above all with what nature could do to us, we have now to worry about what we have done to nature.

According to Vernadsky (1965), "The biosphere is the environment in which we live, it is the 'nature' that surrounds us and to which we refer in common parlance". Lotka (1925) says that "The picture (of Nature) we must keep before us, then, is that of great world engine or energy transformer composed of a multitude of subsidiary units, each separately and all together as a whole, working in a cycle".

"There is no state of nature, such as posited by Rousseau" (Giddens, 1994).

According to *Pope's Essay on Man* (Copleston, 1962):

> All are but parts of one stupendous whole,
> Whose body Nature is and God the soul.

According to T. S. Eliot, "The external Nature is always an accomplice of the illusory reality" (Sen, 1967). But, William Wordsworth proves that Nature is always interesting because of its inherent truth and simple beauty (Sen, 1967). In *The Creative Experiment*, Bowra says that "The whole order of Nature seems to be breaking, and strange sounds and sights testify to the general decomposition" (Rosset, 1948). Stephen

Spender (1950) wanted to substitute the modern civilization with his desired nature of Nature and that is why he said:

Unless, governor, teacher, inspector, visitor,
This map becomes their window and these windows
That shut upon their lives like catacombs,
Break O break open till they break the town
And show the children to green fields, and make their world
Run azure on gold sands, to let their tongues
Run naked into books, the white and green leaves open
History theirs whose language is the sun

What Nature teaches us and what ways of life we should follow will be amply clear from the remark of Kropotkin (1925):

"Don't compete! ---- competition is always injurious to the species, and you have plenty of reasons to avoid it!". That is the tendency of Nature, not always realized in full, but always present. That is the watchword which comes to us from the bush, the forest, the river, the ocean. "Therefore combine --- practice mutual aid! That is the surest means for giving to each and all the greatest safety, the best guarantee of existence and progress, bodily, intellectual, and moral". That is what Nature teaches us; and that is what all those animals which have attained the highest position in their respective classes have done. That is also what man--- the most primitive man -- has been doing; and that is why man has reached the position upon which we stand now….

The Greek sophist Anaxagoras (Stace, 1972) argues that an antithesis is existent between Nature and man. Another Greek sophist Alcidamas of Elaea (Nersesyants, 1986) remarks that "God has set all men free; Nature has made no man a slave". Regarding the relationship between the humans and Nature, the Greek philosopher Aristotle (384-322 BC) says that:

If Nature is to be understood, we must keep in mind certain general points of view…..Nature seeks everywhere to attain the best possible….But if nothing in Nature is aimless or useless, this is not to be interpreted in a narrow anthropocentric spirit. It does not mean that everything exists for the use of man….It is true that, in a certain sense, everything else sublunary is for man. For man is the highest in the scale of beings in this terrestrial sphere….But this does not exclude the fact that lower beings have each its end. They exist for themselves and not for us" (Stace, 1972). He also adds that humanness can not exist apart from human beings, any more than heaviness apart from the heavy object.

Nature can be conceived broadly from three perspectives such as (i) Traditional perspective, (ii) Modern perspective and (iii) New perspective.

2.1. Traditional Perspective of Nature

If human "figure" and its "nose" (recall Gogol's story, called, *The Nose*), which is an inseparable part of the "figure", are respectively likened to the "Nature" and the "human", then traditional perspective shows that "nose" has an independent existence, which means that "nose exists without an owner" and by analogy, it can be said that human society exists without Nature. Traditional perspective is determined by the nature of tradition, traditionalism and traditionalists. Tradition refers to the customs, beliefs, practices, ceremonials, rituals, etc. by which the past can be substituted for the present. Tradition is something, which is given, fixed, or constant, not variable; exogenous, not endogenous; autonomous, not induced; static, not dynamic, and unchallengeable. Tradition is assumed to influence human social life, which can not influence tradition. Human social life is exceptionally dynamic, while tradition is exceptionally static, though it is humanly constructed. Recently tradition is being detraditionalized and reconstructed or reinvented deliberately. The most important characteristic of traditional tradition is that tradition should or can be defended in the traditional way. Like rail lines tradition and Nature go in parallel way. They have vast similarities. The salient features of traditional perspective of Nature are as follows:

If Earth is likened to a model, and if society is treated as endogenous variable (obviously dynamic), then Nature is looked upon as an exogenously and permanently fixed landscape. Symbolically, if Y stands for "index of Nature or Naturalness" measured along the vertical axis and X stands for "index of humanization or socialization" measured along the horizontal axis, then in the two-dimensional diagram, the mathematical function Y = F (X) can be represented by a straight line which is parallel to the X-axis. Nature refers to environment and events which is pregiven independently of human social actions. Nature is devoid of human social spheres. Nature is an autonomously given physical environment that persists only for absorbing social and ecological shocks. It is an external framework for human activity. It is looked at in an instrumental way. It is an external platform of social life and is pregiven and largely unchallengeable. It can or should be returned to its original state by human efforts. The metaphor of Nature as Mother Nature is seriously taken as valid. It is regarded as an object of beauty, separated from human social life. Nature should and can be defended in the natural way as it is "larger than human beings" (Goodin, 1992). It is a non-humanized physical objects or processes (or environment) given independently of human intervention. A return to an independent Nature is advocated by traditionalists. "How shall we live in a world of socialized or lapsed Nature?" is the moral question of traditionalists. Naturalness of Nature can be restored in the natural way. Socialization or humanization of Nature is the only cause of ecological crisis leading to the emerging threat of unsustainability. All humans should become conservative in the conservative way. For the return to natural Nature, traditionalists suggest to follow primitive civilizations and to abandon modern civilizations. As Naess (1972) says that "….. people will be able to live as 'future primitives', recovering ecological diversity as 'dwellers in' the land". Similarly Goldsmith (1988) suggests that it is to

the traditional societies of the past that we must turn for inspiration. Nature should be defended against the inroads of economic expansionism, which threatened its inner harmonies as well as its beauties. The deep ecologists Porritt and Winner seeks to call for a non-violent revolution to overthrow our whole polluting, plundering and materialistic industrial society and, in its place, to create a new economic and social order which will allow human beings to live in harmony with the planet (Dobson, 1990). According to traditionalists, living "close to Nature" implies more harmony with it than living in modern society. Hence primitive ethnoecologies and tribal communities comprising of hunter- gatherers, horticulturists, pastoralists, marginal peasants and the like are placed for admiration. Socialization or humanization of Nature leads to destruction of Nature. Urbanization and globalization backed up by scientific and technological revolution are discouraged. Defending of Nature in the natural way should be paralleled by the defending of tradition in the traditional way. The emphasis on a return to Nature also includes the revival of traditional medicine, substitution of herbs for modern drugs with the exclusion of modern medical methodology. Traditional perspective of Nature has its origin in Cartesian philosophy which indicates the dualistic view of a mind-body actor whose mind chooses between options available to the body in its lived-in situation by a reasoning which transcends the situation, and which then makes the body execute its choice (Bird-David, 1997). Cartesian view also suggests the principles that (a) Everything is revisable, (b) We cannot be sure even about our most cherished ideas and (c) Science is supposed to produce certainties for us. Mastery over Nature means destruction of it, since humanized or socialized Nature is no longer natural by traditional definition. Local, small, diversified and primitive communities are adaptable more gracefully to Nature. So decentralization of cities and reconstruction of ethnoecologies are blissfully encouraged. Conservation of tradition should be coupled with conservation of Nature. So constructions of historical and aesthetical importance should be conserved in the conservative way in the name of conservation of Nature, since these are "larger than humans". Conservation decisions and planning should be undertaken by reference to natural Nature, not humanized or socialized Nature. Scientific and technological civilization should be banned. Nature, ecology and environment are often confused. Villages are more natural than cities.

There are authors who have spoken of the relation between human and Nature, but they have not clarified what Nature and Natural are. For example, according to Smith (1997) for sustainability we require living in peace and comfort within natural limits and preservation of the natural environment in its unaltered state. Living in harmony with natural environment has been suggested by Heang (1997). For sustainability, Posey (1992) has suggested to use the techniques for living in harmony with nature, obtaining favourable results without degrading or exhausting the environment. Living in harmony with surrounding is also the view of Lewis (1992). Man must bring himself into conformity with nature if he wants to exist as part of nature's unity, and must fit his demands to nature's availabilities (Reichel-Dolmatoff, 1976). Living in relative

harmony with nature, and living within the limit fixed as a challenge by nature ensures sustainability (Cavalcanti, 1997).

Traditional perspective of nature is based on the following frameworks, paradigm, doctrines and/or isms:

(a) Possibilism, which means that nature is seen as setting the limits on cultural development, as dictating what is possible (Kroeber, 1939; Stenning, 1957)

(b) Cultural core or cultural ecology of Steward (1955)

(c) Substantivism, which concerns itself with the economy as an instituted process (Sahlins, 1972; Polyani et al., 1957; Halperin, 1988) neglecting the individual.

(d) Formalism, which concerns with the rational individual and the economy at large, as the aggregate of such individuals (Halperin, 1988).

(e) Symbolism or economic symbolism (Sahlins, 1976; Mintz, 1985)

(f) Instrumental rationality.

(g) Old environmental determinism or anthropogeography (Geertz, 1963; Mason, 1896; Huntington, 1924).

(h) Cultural materialism of Harris (1968).

(i) Cultural relativism of Holy and Stuchlik (1981).

(j) Social constructivism of Ingold (1992).

(k) French structuralism of Levi-Strauss (1963).

(l) Cultural economics of Gudeman (1986).

(m) Ecosystem or ecological system (Rapport, 1971).

(n) Cognitive anthropology (Tyler, 1969).

(o) Ethnoecology (Hunn, 1985).

2.2. Modern Perspective of Nature

Giddens (1994) is eulogized for his significant contribution to the modern perspective of Nature. We are living today in a world in which the Nature is being humanized, socialized, remoulded, managed or denatured at an increasing rate. The naturalness of Nature is being faded out and instead, denaturing is taking its place. The humanized or socialized Nature is being substituted for Natural Nature. That is why today's Nature should be designated as humanized, socialized, remoulded or managed Nature. By any criterion, Nature must be conserved, defended or safeguarded. But the way of conservation should be changed. Nature should and can not be defended in the natural way. Consevation of Nature should come from non-natural way. Both the Nature and tradition are alike in the sense that they need defending, since both are disappearing and as tradition should and cannot be defended in traditional way, similarly Nature should and cannot be defended in the natural way. Tradition defended in traditional way means "fundamentalism" which, is dangerous to the society and can arise in all the dimensions of human life.

Nature is going to an end in a parallel way to tradition. Conservation of tradition should effectively be separated from the conservation of Nature. Tradition should be preserved, but the ways of life with which they were associated should and cannot be preserved. For example, we might wish to preserve the local gibbet on account of its history, but not the practice of publicly hanging petty criminals on it (Giddens, 1994). Return to Nature does not necessarily mean the return of the present scientific and technological civilization to the early rude, crude or primitive society, as claimed by traditionalists. Modernists do not believe that communities comprising of hunter- gatherers, horticulturalists, pastoralists, peasants etc. are more natural than modern scientific and technological communities, for their living close to Nature. Because all are human productions and humans are part of Nature. Traditionalist Goddin (1992) argues that "natural products or processes are larger than ourselves or humans". But the modernist Giddens's (1994) counter-argument is that that we need something larger or more enduring than ourselves to give our lives purpose and meaning may be true, but this is plainly not equivalent to a definition of the natural. Mastery over Nature does not mean destroying it. Rather mastery can quite often mean caring for Nature as much as treating it in a purely instrumental or indifferent fashion. Conservation decision and planning should not be undertaken by reference to natural or original Nature, but to humanized or socialized Nature. We have to decide today what nature is and how we should organize our lives in relation to it. The longing for a return to original Nature is a healthy nostalgia, since Nature can never be returned in the natural way. Instead of being concerned above all with what Nature could do to us, we have now to worry about what we have done to Nature. Human constructions such as old buildings, churches, palaces, temples and similar things should be preserved only for their historical importance, not for the fact that they are "larger than ourselves" (Goodin, 1992) and not in the traditional way. We should all become conservative now, but not in the conservative way. Decision about what to conserve or to strive to recover should be determined by reference to denaturing of Nature and detraditionalization of tradition. We should seek to remoralize our lives in a situation where Nature and tradition can be reconstructed in deliberately conscious way. "How shall we live? in a world of lost tradition and socialized Nature" should not be treated as moral despair. We should not start to mistrust science and technology for the lost natural harmonies. Environment, ecology and Nature should not be confused. We can not go back to tradition which is ingrained the community, where tradition is defended in the traditional way. History does not express the will of God but is the result of the active struggles, and creativity, of human beings themselves. The human authorship of history has been hidden by religious dogma and by the dead hand of tradition. The task ahead for humanity is to take hold of its own social development and direct it in a conscious way. We are or can become the masters of our own destiny (Giddens, 1994).

2.3. New Perspective of Nature

The relationship between the Nature and the human society can be likened to the relationship between the Nation State and its government. Government is the subset of the Nation State in the sense that wherever government exists, there is also Nation State, but the converse is not true. Similarly, human society is the subset of Nature, which means that wherever human society exists, Nature follows suit, but wherever Nature exists human society does not exist. Nature should be divided into three zones such as (i) "free-entry zone", (ii) "quasi-entry zone" and (iii) "no-entry zone". So, Nature should be confined not only to the Earth, but a part of the outer space of the universe into which human intrusions are still going on and the rest part of the outer space, which is left untouched till now, should also be included in Nature. Because a part of the outer space of the universe is recently being humanized or socialized in terms of launching of variously artificial satellites and diverse explorations of space to satisfy the human common purposes. Besides, several natural satellites have already been humanized or socialized and many other natural satellites have remained under the queue of humanization or socialization for the common good or bad of the society. Thus, socialized as well as non-socialized parts of the universe should also be included into the new conception of Nature.

Socialization of Nature is a matter of degree, like privatization of national economy, and mathematization of various disciplines or sciences. Though socialization of Nature is going up at an increasing rate, yet full-fledged socialization of Nature is neither physically possible, nor socially desirable. So the moral despair that Nature has fully been denatured or exhausted, or in other words, Nature has ceased to exist, is a sophisticated nostalgia. Unless government ceases to exist how is it possible to realize full-fledged privatization of a national society? Should the national society not decide the degree of privatization consciously for the benefit or desirability of the society? Likewise, while mathematics itself is non-mathematical, since literary or verbal reasoning is still existent in mathematics, is it possible to attain full-fledged mathematization of any science or discipline such as physics, chemistry or the like? If full-fledged socialization of Nature is possible by any means or criterion, then what name should be substituted for remaining non-socialized part of the infinite universe and will diverse non-anthropogenic natural instabilities cease from recurring? And by what name will non-anthropogenic natural instabilities be called? No scientist can predict the upper and lower limits of socialized Nature in the universe despite unprecedented boon of scientific and technological revolution.

Socialization of Nature does not necessary lead to destruction of Nature or natural disequilibrium. All kinds of destructions of nature may be brought about by socialization of Nature, but the converse cannot be true, that is, all kinds of socialization of nature can never be directed to the destruction of Nature. Since human society is a subset of Nature so any kind of socialization of Nature means deliberate intrusion of human intervention into the non-socialized part of the universe. Such human intervention may involve two types of

socialization such as positive and negative. Both the deforestation and reforestation of Nature are the examples of socialization of Nature. But while the former should be called negative socialization, the latter the positive socialization of Nature. If the warfare and the age-old conflicts among diverse fundamentalisms can be ruled out by the conscious and morally optimistic efforts and if at least a few of the lost non-human species can be revived or reinvented by the newly invented technology or methodology, then should these kinds of socialization of Nature be labeled as destruction to Nature? Again the same kind of socialization of Nature may bring about either beneficial or destructive outcome depending upon the politics of the nation state or the scope of socialization. For example, the artificial communication satellite may be used to satisfy socially desirable purpose, say, needs of the scholars and the scientists for internet searching or socially undesirable purpose, say, bringing about future warfare. Since humans rank highest in the scale of species, so they should continue the process of socialization of Nature resorting rationality, morality and consciousness so long as the social instability and ecological instability cannot be wiped out and thereby the newly emerging harmony of socialized Nature can be reestablished, since according to Wilkie (1993) "Human beings retain a moral value, which is irreducible."

The traditionalist insistence that living "close to Nature" (e.g. subsistence living of primitive and tribal communities comprising of hunter-gatherers, horticulturalists, pastoralists, small peasants etc.) is more natural than the living remotely from Nature (e.g. modern scientifically and technologically developed cities). But the question is: which is more natural, the primitive and tribal communities facing large scale poverty, hunger, malnutrition, illiteracy accompanied by high birth and death rates, or the modern developed communities devoid of some of the foregoing social instabilities? In truth, the degree of naturalness of any event or process should not be judged by reference to the natural or crude Nature, rather it should be judged by reference to socialized Nature. Which is more natural, a community "where there is no doctor" (Werner, 1977), or a community where doctor is available for safeguarding the community's health status?

Preservation or conservation of tradition should not be confused with that of Nature. In the socialized Nature, there must be an effective separation between the two. For this confusion may lead to unexpectedly adverse outcomes.

The socialization of Nature is also a natural process, since humans are embedded in the Nature. Through the universal process of evolution, which has not stopped yet, rather is continuing, it has become obvious to the humans that "Man is the highest phenomenon of Nature", according to Greek Stoicism (Copleston, 1962). Owing to humans' supremacy over the Earth, socialization of Nature has become naturally congealed in human nature. By the kind Nature's gentlest boon, humans have been gifted with such qualities as consciousness, morality, rationality etc. by which they can socialize the Nature in the positive direction to bring about a transition to the dynamically social stability and ecological stability, given the constant threat

of exogenously determined non-anthropogenic natural instability. The Greek philosopher Heracleitus (535-475 BC) said that "Everything in the universe has in it its own opposite" (Stace, 1972). Further according to the principle of antinomy of terminology, concepts arise in science in pairs---every phenomenon must have a corresponding anti-phenomenon and every process must have a process with its opposite polarity. Thus, we cannot speak of the Nature or Natural without denoting its opposite---- anti-Nature or anti-Natural. Traditionalists backed up by fundamentalism insist that the opposite of Nature, Natural, Naturing or Naturalization is society (humanity), social (human), socializing (humanizing) or socialization (humanization). But they can be reminded that the latter is embedded in the concept of the former.

3. Interpretation of Stoic Ethical Precept

According to Stoics, the end of life is to attain happiness, which is possible in the Natural life or life according to Nature, which means the agreement of human action with the law of Nature. For man to conform himself to the laws of universe in the wider sense, and for man to conform his conduct to his own essential nature, that is, reason, is the same thing, since universe is governed by the law of Nature. While earlier stoics such as Zeno, Cleanthes, Chrysppus, et al. thought of Nature which man should follow, rather as the Nature of the universe, later Stoics such as Seneca, Epictetus, Aurelius, et al. tended to conceive Nature from a more anthropological point of view. The conflicting senses of the Stoic ethical maxim "live or life according to Nature" are as follows:

By Nature, Cynics mean rather the primitive and instinctive, and so life according to Nature implies a deliberate flouting of the conventions and traditions of civilized society, a flouting that externalizes itself in conduct that is eccentric and not infrequently indecent.

According to Stoics, life according to Nature indicates life according to the principle that is active in Nature. The ethical end of life lies in submission to the order and arrangement of the universe. Man is endowed with reason, the faculty which gives him his superiority over the brute and so for man life according to Nature is rightly understood to mean life according to reason. The end of life is a life which follows Nature, whereby is meant not only our own nature, but the Nature of the universe, a life wherein we do nothing that is forbidden by the universe i.e. by right reason. The ethical teaching of the Stoics thus declares that happiness is a life according to Nature, while a life according to Nature is a life according to right reason.

By "live or life according to Nature", the traditional perspective indicates that humans should live in accordance with the non-socialized, crude or natural Nature as primitive and tribal communities do, for maintaining globally ecological equilibrium or sustainability, irrespective of social sustainability or unsustainability.

But modern perspective expresses just opposite view, which means that humans as the part of Nature must live in accordance with the humanized or socialized Nature and socialization of Nature can not be stopped so long as humans survive in the Earth as the highest creature.

Stoic Seneca's ethical precept "live or life according to Nature" has been renamed by the new paradigm as "sustainability" provided that the negative autonomous socialization of Nature can be at least compensated (or overcompensated, not undercompensated) by the positive autonomous socialization backed up by human trinity "rationality, consciousness and morality", treating such socialization as a sustained and dynamic process. This means that positive accommodating socialization of Nature must reproduce "socialized sustainability" (obviously renewed) on the assumption that society itself has been "optimally socialized" without any kind of fundamentalism.

4. Should Society Sustain Stoic Ethical Precept?

The Northern scholar Smith (1997) claims that "the notion of environmentally sustainable development was promoted in the 1970s most prominently by Herman Daly (1972) and 'sustainable development' as a concept is a product of the North". But, retrospective evidences reveal that 1970s-Northern concept of "sustainability" is congealed in the ancient Greek Stoic ethical precept "live or life according to Nature". So Greek Stoicism should be admired for the invention of the present day concept of sustainability. Regarding the foregoing question indicated by this section my suggestion is that the world in which today we live should be labeled as a "runaway world" (Giddens, 1994), which should be characterized by "global unsustainability syndrome" caused by increasing socialization of Nature, in which "negative socialization" is partly offset by the "positive socialization" (Konar, 2013) of Nature. In such a situation, we should stoop to the Stoic ethical precept under the following preconditions:

(i) The first precondition to be remembered is that our Nature is the humanized or socialized Nature.

(ii) Socialization of Nature can never be stopped. Its sustenance and dynamism are consistent with the sustained process of universally natural evolution and humanly induced various revolutions.

(iii) Along with rationality, consciousness and intelligence, humans are also endowed with moral values by which they should be directed along such a pathway of continuous socialization of Nature so that the "negative socialization" (Konar, 2013) can be overcompensated by the "positive socialization" (Konar, 2013) of Nature.

5. Concluding Comments

Following Maxim Gorky (Borisov, 1986) who in praise of human reason, science and technology wrote: "In nature there is nothing more miraculous than the human brain, more amazing than the process of thinking, more precious than the fruits of scientific research", it may be concluded that Stoic ethical dictum " Live or life according to Nature" should be sustained for the potential transition to the world of secularly renewed sustainability indicated by ecological (stability) sustainability coupled with social (stability) sustainability, given the exogenously determined non-anthropogenic Natural instability, through the socialization of Nature only in rational, conscious and moral pathway, though the uncertainty expressed by Georgescu- Roegen (1971) that "no social scientist can possibly predict what kinds of social organization mankind will pass in its future" may be unavoidable and unchallengeable.

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In This Issue

The Embeddedness of Depression in the Simple Keynesian Model

Arup Kanti Konar [1]

Abstract

The sole objective of this very short paper was to disclose that depression is ingrained in the simple Keynesian model of hydraulic Keynesianism. Depression occurs in the Simple Keynesian Model because of the fact that the rate of change (growth) of saving is greater than the rate of change (growth) of investment. Depression can be cured, or prosperity can be realized if the rate of change (growth) of saving is equal to or less than the rate of change (growth) of investment.

Keywords **: Keynesianism, consumption, saving, investment, stability**

JEL Classification Codes : E12, E21, E32, E63

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The sole objective of this very short paper is to disclose that depression is ingrained in the simple Keynesian model of hydraulic Keynesianism, coined by Alan Coddington (1976, 1983). It is noteworthy that the other two types of Keynesianism are (a) fundamentalist Keynesianism and (b) disequilibrium Keynesianism or reconstituted reductionism. Further, it can be pointed out that the other three variants of hydraulic Keynesianism are : (a) special Keynesian model, (b) IS-LM Keynesian model or more general Keynesian model, and (c) generalized or complete Keynesian model.

Hydraulic Keynesianism was devised by Hicks (1937), Meade (1937), Samuelson (1939a, 1939b, 1946, 1947, 1948), Harrod (1937), Hansen (1936a, 1936b, 1938, 1941, 1947, 1951, 1953), Smith (1956), and so forth.

The simple Keynesian model is based on the following three equations, which have been translated into Figure 1.

(1) $S = S_a + S(Y)$ such that $S_a < 0$ and $1 > S'(Y) = MPS > 0$

(2) $I = I_a + I(Y)$ such that $I_a > 0$ and $0 < I'(Y) = MPI < S'(Y) = MPS$

where,

$MPS \equiv$ marginal propensity to save (slope of saving function) and $MPI \equiv$ marginal propensity to invest (slope of investment function).

(3) $S = I$ (equilibrium condition of commodity market)

The point elasticity of S with respect to Y of the S function $= E_{SY} = (dS/S)/(dY/Y) = (dS/dY)/(S/Y) = S'(Y)/(S/Y) = MPS/APS > 1$, since $MPS > APS$, where $APS = S/Y$. So, $E_{sy} > 1$, which means that $dS/S > dY/Y$. This implies that the rate of change (growth) of saving is greater than the rate of change (growth) of income.

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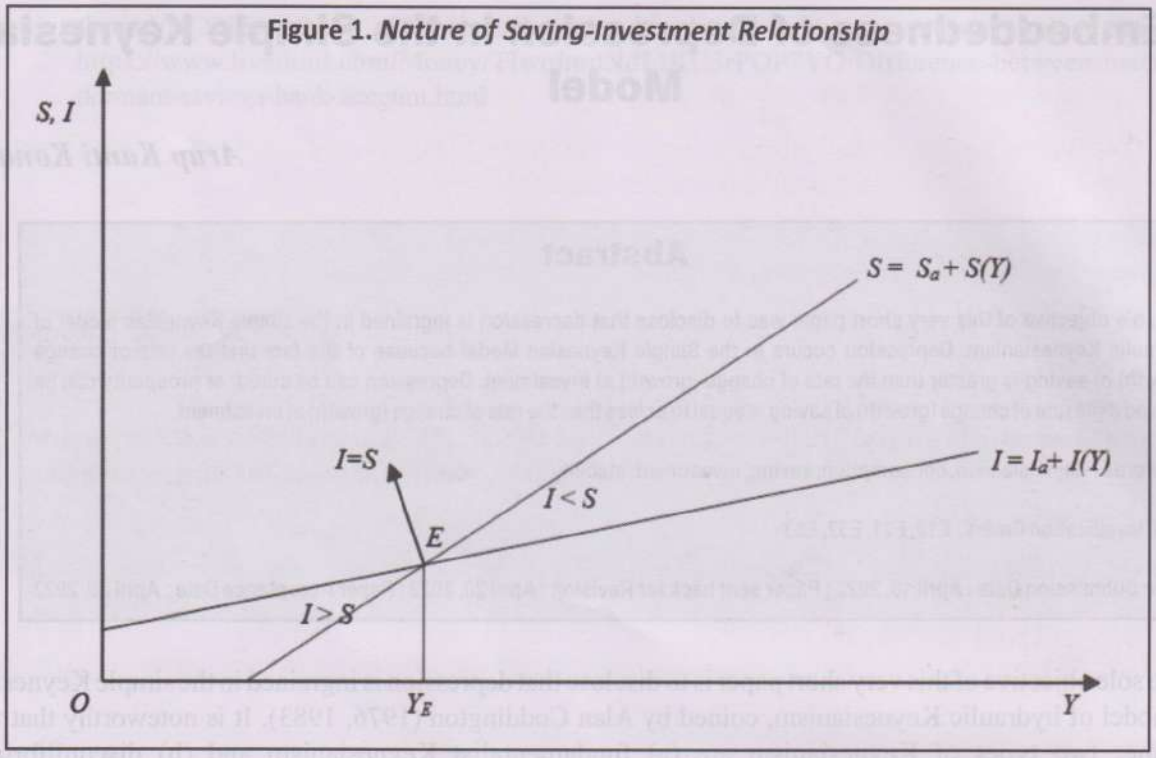
Figure 1. ***Nature of Saving-Investment Relationship***



On the other hand, the point elasticity of I with respect to Y of the I function = E_{IY} = $(dI/I)/(dY/Y)$ = $(dI/dY)/(I/Y)$ = $I'(Y)/(I/Y)$ = $MPI/API < 1$, since $MPI < API$, where $API = I/Y$. So, $E_{IY} < 1$, which means that $dI/I < dY/Y$. This implies that the rate of change (growth) of investment is less than the rate of change (growth) of income.

Thus, at the point of equilibrium denoted by E in Figure 1, though $S = I$ and $MPS > MPI$, yet we have: $E_{sy} > E_{IY}$, which means $dS/S > dI/I$, that is, the rate of change (growth) of saving is greater than the rate of change (growth) of investment, which is the vital condition of depression. Hence, in the developed capitalist economy, depression is inevitable owing to the fact that $MPS > MPI$ and $dS/S > dI/I$. Depression can be cured, or prosperity can be realized if $dS/S = dI/I$, which is the condition of stability, and it implies that the rate of change (growth) of saving is equal to the rate of change (growth) of investment or, $dS/S < dI/I$, which is the condition of prosperity and it implies that the rate of change (growth) of saving is less than the rate of change (growth) of investment.

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Aerofilum fasciculatum gen. nov., sp. nov. (Oculatellaceae) and *Euryhalinema pallustris sp. nov.* (Prochlorotrichaceae) isolated from an Indian mangrove forest

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Abstract

Two novel cyanobacteria (AP3 and AP3b) with thin cells and simple morphology were isolated from two islands of the Indian Sundarbans. The 16S rRNA phylogeny data revealed the distinct lineage of AP3b which was nearest to the clade incorporating the genus *Oculatella* and *Tildeniella*. Strain AP3 shared a common ancestor with the species *Euryhalinema mangrovii*. Additionally, the novel 16S rRNA gene sequences of strains AP3 and AP3b showed similarities about 98% and 93% respectively compared to those of established genera or species to which they were phylogenetically related. Furthermore, the folding patterns of semi-conservative structures like D1-D1', Box-B and V2 helices of 16S-23S ITS region for both strains AP3 and AP3b displayed significant variations and uniqueness when compared with their respective reference strains (*Euryhalinema mangrovii* for AP3 and all the genera of Oculatellaceae for AP3b). Strain AP3 shared similar morphological features with its reference strain which confirmed its inter-species relationship. The diagnostic features of AP3b including the presence of necridic cells, aerotopes and a cluster-like growth pattern were found to be very contrasting. Altogether, these results substantiated the establishment of strain AP3b as a novel mono-specific genus named *Aerofilum fasciculatum* and strain AP3 as the second novel species under the genus *Euryhalinema*, referred to as *Euryhalinema pallustris*.

Keywords: *Aerofilum, Euryhalinema,* cyanobacteria, 16S-23S ITS, 16S rRNA, Sundarbans

Introduction

Cyanobacteria are well diversified group of organisms and undergo speciation. One of the main governing factors in their evolution is horizontal gene transfer which takes place among related cyanobacterial populations (Tooming-Klunderud *et al.* (2013). This evolution is further indicated by substantial variations in the morphology and ecophysiological characters (Komarek & Anagnostidis 2005). The speciation event promoted by the phenomenon of horizontal gene transfer depends on the environment of the organism and the degree of shared genes among the inhabiting species increases with increase in the extremities of the environment (Fuchsman *et al.* 2017). The intertidal regions of the world's largest mangrove forest, the *Sundarbans* are characterized by periodic changes in the physical parameters like water salinity, temperature, pH, carbon dioxide levels that can vary widely (Neogi *et al.* 2016). The biofilm

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forming cyanobacteria residing in this intertidal zone are adapted to this variable poikilotrophic environment, leading to alterations in their genomes which culminate into eventual diversification as novel organisms (Debnath *et al.* 2017). Recent investigations on the cyanobacterial diversity of the Indian *Sundarbans* has resulted in the description of novel genera and species like *Leptolyngbya indica* (Debnath *et al.* 2017:105), *Oxynema aestuarii* (Chakraborty *et al.* 2018:37), *Euryhalinema mangrovii* (Chakraborty *et al.* 2019:70) and *Leptoelongatus litoralis* (Chakraborty *et al.* 2019:70). These findings suggest that biofilm-forming cyanobacteria of the intertidal regions of the Indian Sundarbans hold the potential for further discovery of novel cyanophyta.

Cyanobacterial systematics is currently founded on the polyphasic approach to taxonomy where the primary systematic position is determined on the basis of phylogeny established by the genetic markers, mainly 16S rRNA gene, located in the DNA sequence, supported by ecological variations and specific shared and derived morphological as well as ultrastructural features (Komarek *et al.* 2014). Contemporary literature demonstrates that growth of phylogenetic trees representing the evolutionary relationships among the members of cyanobacteria is occurring continuously as evidenced by descriptions of many new genera and species. Out of eight orders described by Komarek *et al.* (2014), Synechococcales is challenging because this order consists of members which are phylogenetically intermixed. Leptolyngbyaceae and Prochlorotrichaceae are believed to be the most polyphyletic families in this order. Sequences of many strains submitted in the National Center for Biotechnology Information (NCBI, http:/ www.ncbi.nlm.nih.gov/genbank/) database were named as "*Leptolyngbya* species" or "Leptolyngbyaceae", although they were evolutionarily distinct from the *Leptolyngbya* generitype (Becerra-Absalon *et al.* 2018, Mai *et al.* 2018). These inappropriate names resulted in a polyphyly within the *Leptolyngbya* genus. Although Leptolyngbyaceae and Prochlorotrichaceae families were subjected to many taxonomic revisions, further taxonomic reconsiderations following the polyphasic approach is required for many of these misnamed strains. Over the last ten years, many new generic entities were separated from the polyphyletic genus *Leptolyngbya*. These novel genera include *Phormidesmis* (Komarek *et al.* 2009: 53, Turichhia *et al*. 2009: 179), *Nodosilinea* (Perkerson *et al*. 2011: 1404), *Haloleptolyngbya* sp. (Dadheech *et al.* 2012: 272), *Chroakolemma* sp. (Becerra-Absalon *et al*. 2018: 204) and *Albertania* sp. (Zammit, 2018: 483). Furthermore, Mai *et al.* (2018) proposed a family-level taxonomic reorganization on the basis of the polyphasic approach which eventuated into the division of family Leptolyngbyaceae into four monophyletic families comprising of two novel families Oculatellaceae and Trichocoleaceae and two previously-described families Leptolyngbyaceae and Prochlorotrichaceae. Mai *et al.* (2018) proposed six new genera under the novel family Oculatellaceae containing 14 new species which established the family Leptolyngbyaceae as a well-defined and monophyletic clade. Moreover, the family Prochlorotrichaceae was redefined by Mai *et al.* (2018) containing the genera *Prochlorothrix*, *Nodosilinea*, *Halomicronema* and some members without any specific generic identity. Chakraborty *et al.* (2019) described two novel monospecific genera *Euryhalinema mangrovii* and *Leptoelongatus litoralis* which shared well-supported molecular phylogenetic relationships with the other genera of Prochlorotrichaceae. Family-level taxonomic analyses based on phylogenetic relationship among the families under the Synechococcales order had established a monophyletic trend. Nevertheless, further revision for the establishment of monophyletic affiliation at the inter-generic level is essential owing to the presence of several unidentified generic entities. Most of the filamentous taxa in the Synechococcalean order have a simple morphology but they are genetically very divergent. Becerra-Absalon *et al.* (2018) specified the requirement of more in-depth taxonomical evaluation for the description of new genera and naming a genus is consequently followed by description of new species under it. For instance, many genera like *Oculatella, Oxynema, Nodosilinea* were regularly revised and new species were subsequently added thus increasing the resolution of the phylogenetic clade. In this context, all the strains with ambiguous identity and nomenclature like "*Leptolyngbya* species" (excluding the clade of *Leptolynbya sensu stricto*) and "*Calothrix* sp. 96/26 LPP3" which are till date incompletely studied, requires to be examined thoroughly. This could be a resourceful approach for the establishment of complete monophyletic groups of genera under a family (Becerra-Absalon *et al.* 2018, Chakraborty *et al.* 2019).

This article contributes to the alpha-level taxonomy of the family Oculatellaceae and Prochlorotrichaceae. The present study aimed to investigate two strains, AP3 and AP3b isolated from the mangrove forest of the Indian Sundarbans by applying the polyphasic approach to taxonomy. Through a combination of morphological, ultrastructural and molecular data as well as ecological considerations we propose that strain AP3 should be recognized as the second novel species of the newly established genus *Euryhalinema* (Chakraborty *et al.* 2019) as *Euryhalinema pallustris sp. nov.* while strain AP3b should be accepted as a monospecific novel genus *Aerofilum fasciculatum* gen. nov.

Materials and methods

2.1 Study site, collection and maintenance of strains Strains AP3 and AP3b were obtained from the biofilms present on the soil surface of the Sagar and Lothian islands of the Indian Sundarbans respectively (Fig. 1). The physical characteristics of the collection site were detailed by Pramanik *et al.* (2011). The isolated cyanobacteria were purified and propagated in the laboratory and were checked in every stages of their growth period for the stability of their phenotypic characteristics at monthly intervals. No changes were observed during the successive sub-cultures. The pure cultures of the cyanobacteria were maintained in ASN III liquid medium (Rippka *et al.* 1979) as well as in solid medium. The cyanobacteria were cultivated in a controlled environment having fluorescent irradiance (50 µmol photons m^{-2} s^{-1}) with 12 : 12 hrs light : dark photoperiod at 25 ± 1 °C (Chakraborty *et al*. 2018, Chakraborty *et al.* 2019). Isolate AP3 having accession number MCC 3172 and AP3b bearing accession number MCC 3478 were deposited in the Microbial Culture Collection (MCC), India where they are being cryopreserved.

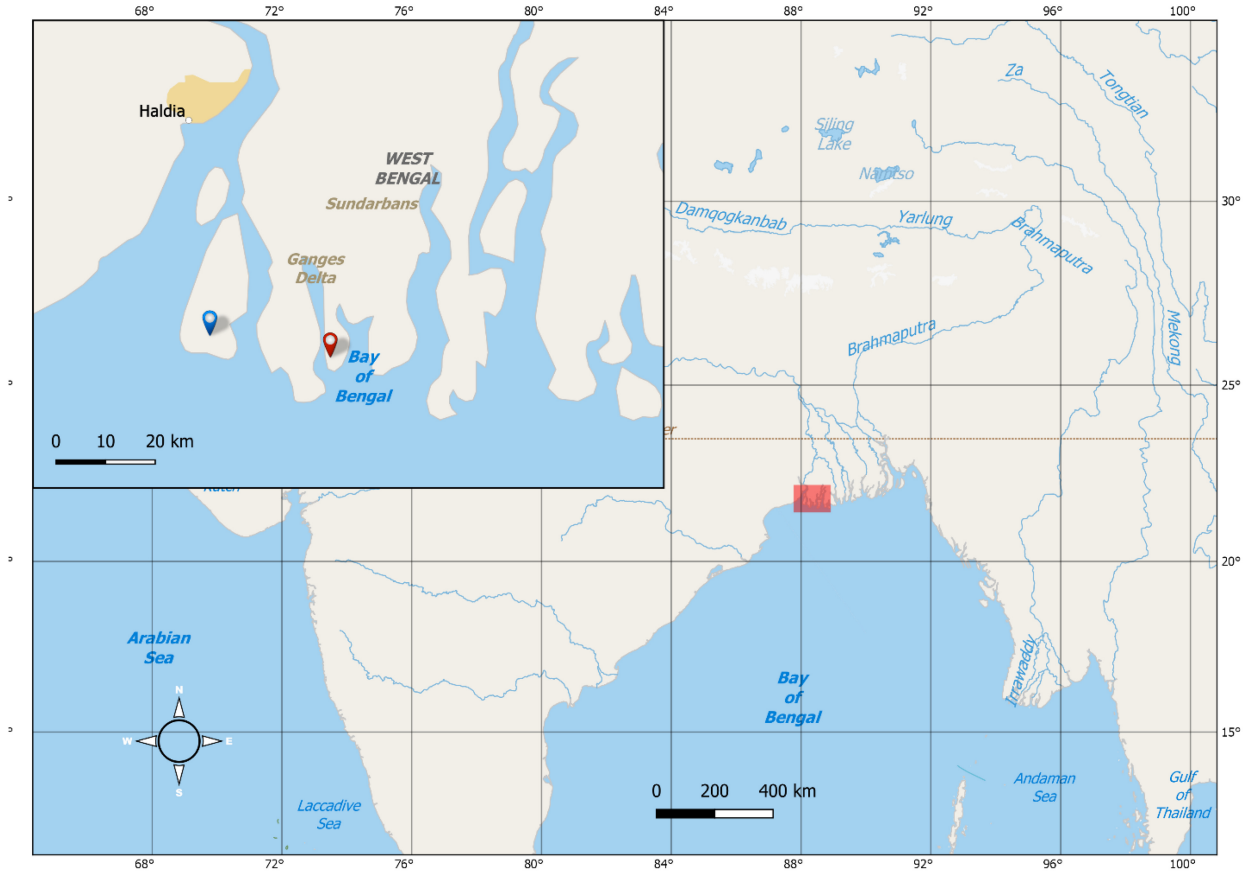

FIGURE 1. Map showing the location of sample collection sites of the Sundarbans forest located in the state of West Bengal, India. Samples were collected from the Sagar and the Lothian island of Indian Sundarbans. Blue pin = Sagar island; Red pin = Lothian island

2.2 Light microscopy Morphological examination was carried out using a light microscope (Model DM750; Leica Microsystems, Buffalo Grove, USA) under 1000X magnification. Photomicrographs of the filaments from the exponential as well as stationary phases of the life cycle of AP3 and AP3b were acquired by a camera (ICC50 HD) attached to the microscope (Chakraborty *et al*. 2018, Chakraborty *et al.* 2019). Cellular dimensions were recorded by the aid of auxiliary software (LAS-EZ, Leica Microsystems) available with the microscope.

2.3 Scanning electron microscopy One milliliter suspension of fresh filaments of test strains (AP3 and AP3b) were concentrated by centrifugation at 8000 rpm (Eppendorf 5810R, rotor F-34-6-38, Hamburg, Germany). The cells were fixed in 3% glutaraldehyde for 2 hrs and successively washed in distilled water. Cells were dehydrated applying enhancing concentrations of ethanol from 30% for 15 min to 100% for 60 min. The samples were ultimately dried at the critical point and the grids were examined under a scanning electron microscope (Jeol JSM-6700F, Jeol, Tokyo, Japan) following Chakraborty *et al*. (2018) and Chakraborty *et al*. (2019).

2.4 Transmission electron microscopy About 1 ml suspension of fresh cyanobacterial cells from 8-10 days old cultures of the strains under examination were centrifuged at 8000 rpm (Eppendorf 5810R, rotor F-34-6-38, Hamburg, Germany). The pellet was washed carefully in distilled water and successively pre-fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.8) for 5-6 hrs at 4 °C. The samples were rinsed in 0.1 M phosphate buffer and the surplus fixative was washed off with the buffer. Post fixation of the cells with osmium tetroxide (1% solution) was carried out for 60 minutes. Subsequently, cells were dehydrated by passing through enhancing concentrations of ethanol. Afterwards, samples were infiltrated and embedded in Araldite CY 212 (Agar Scientific, Stansted, UK) for incising of sections. The resin blocks were polymerized by heat treatment at 50 °C overnight and then subjected to a second treatment at 60 ºC for 2 days. An ultramicrotome was applied to cut thin sections of the samples. Sections were contrasted with uranyl acetate and lead citrate and examined under a TECNAI G20 transmission electron microscope (FEI, Eindhoven, Netherlands) as described in Chakraborty *et al*. (2018) and Chakraborty *et al*. (2019).

2.5 DNA extraction, PCR amplification and sequencing DNA was extracted from the exponential phase of growth of the axenic cultures of the test strains (AP3 and AP3b) using Gene JET™ Genomic DNA Purification Kit (Cat. No. K0721, Thermo Scientific, Waltham, USA) following the manufacturer's instructions. The DNA extracts were stored at -20 °C. Polymerase Chain Reaction (PCR) was applied to amplify the partial 16S rRNA region and the associated 16S-23S ITS region as described in Chakraborty *et al.* (2018) and Chakraborty *et al*. (2019). Briefly, we employed cyanobacterial specific forward primer CYA106F (5'-CGGACGGGTGAGTAACGCGTGA-3') (Nubel *et al*. 1997) and universal reverse primer 1492R (5'-ACCTTGTTACGACTT-3') (Lane 1991) for the amplification of 16S rRNA gene. Forward primer 16SF (5'-TGTACACACCGGCCCGTC-3') and reverse primer 23SR (5'-CTCTGTGCCTAGGTATCC-3') as designed by Iteman *et al*. (2000) were applied for the amplification of the 16S-23S ITS region. The program used for the PCR reaction using primers CYA106F and 1492R was: 94 °C for 5 minute, 94 °C for 1 minute, 72 °C for 2 minutes (30 cycles), followed by a 10 minute extension at 72 °C (Chakraborty *et al.* 2018, Chakraborty *et al.* 2019). Amplified PCR products were stored at 4°C. The PCR program for amplification of the 16S-23S ITS regions were: initial denaturation at 95 °C for 5 min thereafter 30 cycles at 95 °C for 30 sec; 58 °C for 15 sec; 72 °C for 40 sec and ultimate elongation step at 72 °C for 5 min (Chakraborty *et al.* 2018, Chakraborty *et al.* 2019). Reactions were carried out using Mastercycler Nexus Gradient PCR machine (Eppendorf, Hamburg, Germany). PCR products were detected by standard agarose gel electrophoresis with ethidium bromide staining. The PCR products obtained for 16S rRNA and ITS regions were cloned into plasmids containing the sites for their respective primers on either side of the insert site using InsTAclone™ PCR cloning kit (Cat. no. K1213, K1214, Thermo Scientific, Waltham, USA). The commercial vector used for insertion of the sequence was pCR 2.1 (Life Technologies, Invitrogen, USA). Competent *E. coli* DH5α cells were used for the transformation. Colonies were selected by blue-white screening method and the plasmid DNA was purified and obtained from the resultant clones using Thermo Scientific GeneJET Plasmid Miniprep Kit (Cat. no. K0502, K0503, Waltham, USA). Clones containing PCR products were digested with EcoRI and Hind III enzyme and run on an electrophoresis gel to estimate the size of the inserts. Four clones for strain AP3b and three for strain AP3 were selected for sequencing. Finally, sequencing was performed using an automated DNA sequencer (Genetic Analyzer 3500xL, Applied Biosystems, Waltham, USA) as described in Chakraborty *et al.* (2018) and Chakraborty *et al.* (2019). The sequences of 16S rRNA and 16S-23S ITS regions of all the clones of the isolates AP3 and AP3b obtained were checked for its authenticity and reliability by determining the quality control values and any chimeric sequence, if present, using online software tool DECIPHER (http://www2.decipher.codes). Sequences were subsequently submitted to GenBank and their accession numbers are presented in Table 1.

TABLE 1. Gene sequences of the cyanobacterial test strains submitted in the NCBI database with their accession numbers.

Strain Id	NCBI Accession numbers	
	16S rRNA	16S-23S ITS
Euryhalinema pallustris AP3 clone 1	MT310717.2	MT310719.2
Euryhalinema pallustris AP3 clone 2	MT947790	MT943581
Euryhalinema pallustris AP3 clone 3	MT947791	MT943582
Aerofilum fasciculatum AP3b clone 1	MT310718.3	MT310720.2
Aerofilum fasciculatum AP3b clone 2	MT947792	MT943583
Aerofilum fasciculatum AP3b clone 3	MT947793	MT943584
Aerofilum fasciculatum AP3b clone 4	MT947794	NA

2.6 Examination of the 16S rRNA gene sequence and phylogenetic analysis The gene sequences of all the clones of the isolates under investigation (AP3 and AP3b) were examined by using Basic Local Alignment Search Tool (BLAST) and compared with the other sequences available in the robust database of the National Center for Biotechnology Information (NCBI, http:/www.ncbi.nlm.nih.gov/genbank/). Additional sequences were also obtained based on the classification criteria involving the sequences of many recently described genera of the family Oculatellaceae as well as Prochlorotrichaceae within the order Synechococcales. A consensus phylogenetic tree was constructed on the basis of the similarity values obtained in the BLAST hits and the sequences selected based on the classification criteria. The taxa used in the construction of phylogenetic tree included in total 115 OTUs and *Gloeobacter violaceus* was selected as the outgroup. The multiple sequence alignment of these OTUs was performed in the Clustal W program (Larkin *et al.* 2007). Bayesian Inference (BI), and Maximum Likelihood (ML) analyses were performed using 16S rRNA gene sequences of the 116 OTUs with maximum of 1778 characters containing nucleotides and indels. Maximum Likelihood (ML) tree was reconstructed using MEGA program package version 6.0 (Tamura *et al.* 2013) employing Kimura's two parameter model of sequence evolution with gamma distributed evolutionary rates and an estimated proportion of invariable sites. Bootstrap values to test the robustness of the phylogenetic tree for ML analysis were set to 1000 replications. Bayesian Inference analysis was executed by submitting the alignment to MrBayes (Ronquist *et al.* 2012) on XSEDE (3.2.6) available on CIPRES Science Gateway v.3.3 (Miller *et al.* 2010) applying a GTR+G+I model of nucleotide substitutions. Two independent runs with four chains were executed and each of the two runs was simultaneously conducted for 30 million Markov Chain Monte Carlo (MCMC) generations. Temperature was empirically set to 0.2 and permitted the sampling of trees after every 500 generations. The Estimated Sample Size (ESS) of this analysis exceeded 300 for all parameters which are usually considered acceptable by all phylogeneticists (Drummond *et al.* 2006). The final average standard deviation of split frequencies was lower than 0.008. The Potential Scale Reduction Factor (PSRF) value for all the parameters was 1.00 signifying the statistical attainment of the convergence of MCMC chains (Gelman & Rubin 1992). The first 500 trees were rejected as burn-in phase and a 50% majority rule consensus tree was evaluated comprising posterior probabilities. The resultant tree was visualized in the FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree) software. . An array of 16S rRNA and ITS divergence rates among the sequences was studied by the computation of uncorrected p-distance for 16S rRNA and ITS regions in MEGA 6.0 and the output was used to calculate similarity matrix (as percentage) [100×(1-p)] for 16S rRNA data and a dissimilarity matrix (as percentage) (100×p-distance) for the ITS data. 16S rRNA sequences for both the studied strains were also utilized for examining the conserved regions and the secondary structures of its various helices following Rehakova *et al.* (2014) and to verify the quality of sequences for our strains by aligning them with other reference strains identified by Rehakova *et al.* (2014). Sequences of the conserved regions were folded in M-fold web server (Zuker 2003).

2.7 Analysis of 16S - 23S ITS secondary structures The sequences of 16S - 23S ITS regions were also utilized for the taxonomic resolution of the strains under investigation, AP3b and AP3 at the genus and species levels respectively. The complete ITS sequences for both strains were aligned with their respective reference strains (AP3 with its reference strain *Euryhalinema mangrovii* and AP3b with the other related genera belonging to the family Oculatellaceae) and all the variable as well as conserved regions were identified. The complete ITS sequences of the reference strains were obtained from the NCBI. The constituent ITS regions like D1-D1' helix, Box- B, V2 and V3 helices were folded for AP3b and D1-D1' helix, Box- B and V2 helices for AP3 by operating the M-fold web server, version 2.3 (Zuker 2003) and redrawn employing Adobe Illustrator 2020. The secondary structures were created applying ideal conditions with the temperature fixed at default value 37 ˚C and the structure assigned as untangle loop fix. Additionally, the sequence length of the various constituent regions of the ITS were determined and compared with their respective reference strains following Johansen *et al.* (2011). The sequence lengths of the various constituent regions of ITS of the reference strains were obtained from Chakraborty *et al.* (2019) and Mai *et al.* (2018).

Results

3.1 Morphological characterization When provided with the ASN III medium, strains under investigation (AP3 and AP3b) displayed moderate growth in the Erlenmeyer flasks. Strain AP3 formed a uniform, thin mat-like greenish biofilm attached to the surface of the flask. Strain AP3b grew in clusters, appearing as fascicles (Komarek & Anagnostidis 2005) rather than forming a consistent biofilm attached to the surface. Strains under investigation (AP3 and AP3b) were characterized by light microscopy (Fig. 2). Trichomes of AP3b appeared in clusters. Individual

filaments consisted of a facultative sheath. Trichomes were isopolar, uniseriate, unbranched, thin having cells slightly longer than their width, cell length ranged from 1.4 - 2.1 µm and width 0.9 - 1.1 µm. Cells showed fine constrictions at their cross walls (Fig. 2c & 2d). Trichomes were cylindrical, immotile, did not contain any heterocyte or akinete. Small disintegrated filaments were found as hormogonia which help in propagation of the cyanobacterial cells. Cells of strain AP3 possessed dimensions 1.1 - 1.6 µm (length) and 0.4 - 0.5 µm (width) (Fig. 2a & 2b). Cells of the filaments were much longer than their width. General features were alike strain AP3b except that isolate AP3 was not covered by any mucilage sheath. Scanning electron microscopy (Fig. 3) also demonstrated features that were similar to that revealed by light microscopy. Presence of a facultative mucilaginous sheath covering the trichome of strain AP3b was confirmed by the scanning electron microscopy (Fig. 3e) while Fig. 3b shows the presence of a necridic cell (sacrifying cell) (Komarek & Anagnostidis 2005) separating from the terminal part of the parent trichome to form hormogonia.

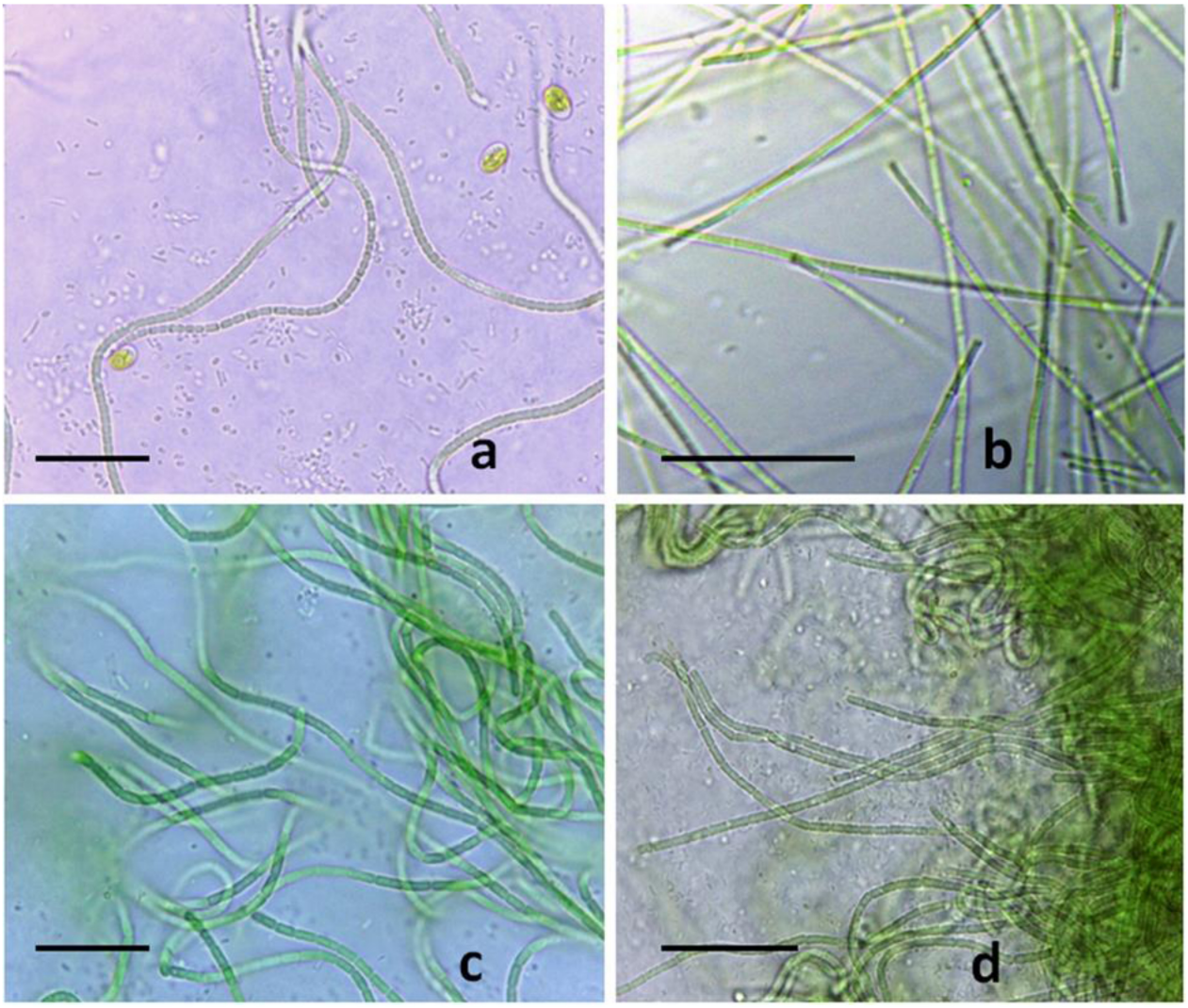

FIGURE 2. Light microscopy of strain AP3 and AP3b (non-axenic cultures). **a** and **b**. Microphotographs showing the filaments of strain AP3. **c** and **d**. Microphotographs showing the filaments of strain AP3b. Filaments observed in **a** & **c** were from exponential phase and in **b** & **d** were from stationary phase of the growth period for the respective strains. Scale = 10 µm

The closest species to be compared with AP3 on the basis of phylogenetic data as well as morphometric analysis was the mono-specific genus *Euryhalinema mangrovii* under the family Leptolyngbyaceae recently described by us (Chakraborty *et al.* 2019). The studied isolate AP3 showed high affinity with this genus in the morphological characters. Similarly, strain AP3b was found to be most closely related to *Oculatella atacamensis* as well as *Tildeniella torsiva* belonging to a recently described family Oculatellaceae (Mai *et al.* 2018). Therefore, a morphometric comparison amongst AP3 and *Euryhalinema mangrovii* (Table 2) and a comparative account of morphological features of AP3b in contrast with the phylogenetically closest relative *Oculatella atacamensis* as well as other related genera of

family Oculatellaceae are presented (Table 3). Table 2 depicts that the strain AP3 showed most of the morphological features similar to its reference *Euryhalinema mangrovii* except their collection site which was two separate islands, geographically 10 kms apart. Table 3 showed diagnostic morphological features of strain AP3b which were distinct from the other genera compared like the presence of aerotopes and fascicular growth pattern while some features like cellular dimensions, cellular constrictions, cell shapes and apical cell morphology in AP3b were shared with the other genera of the family Oculatellaceae.

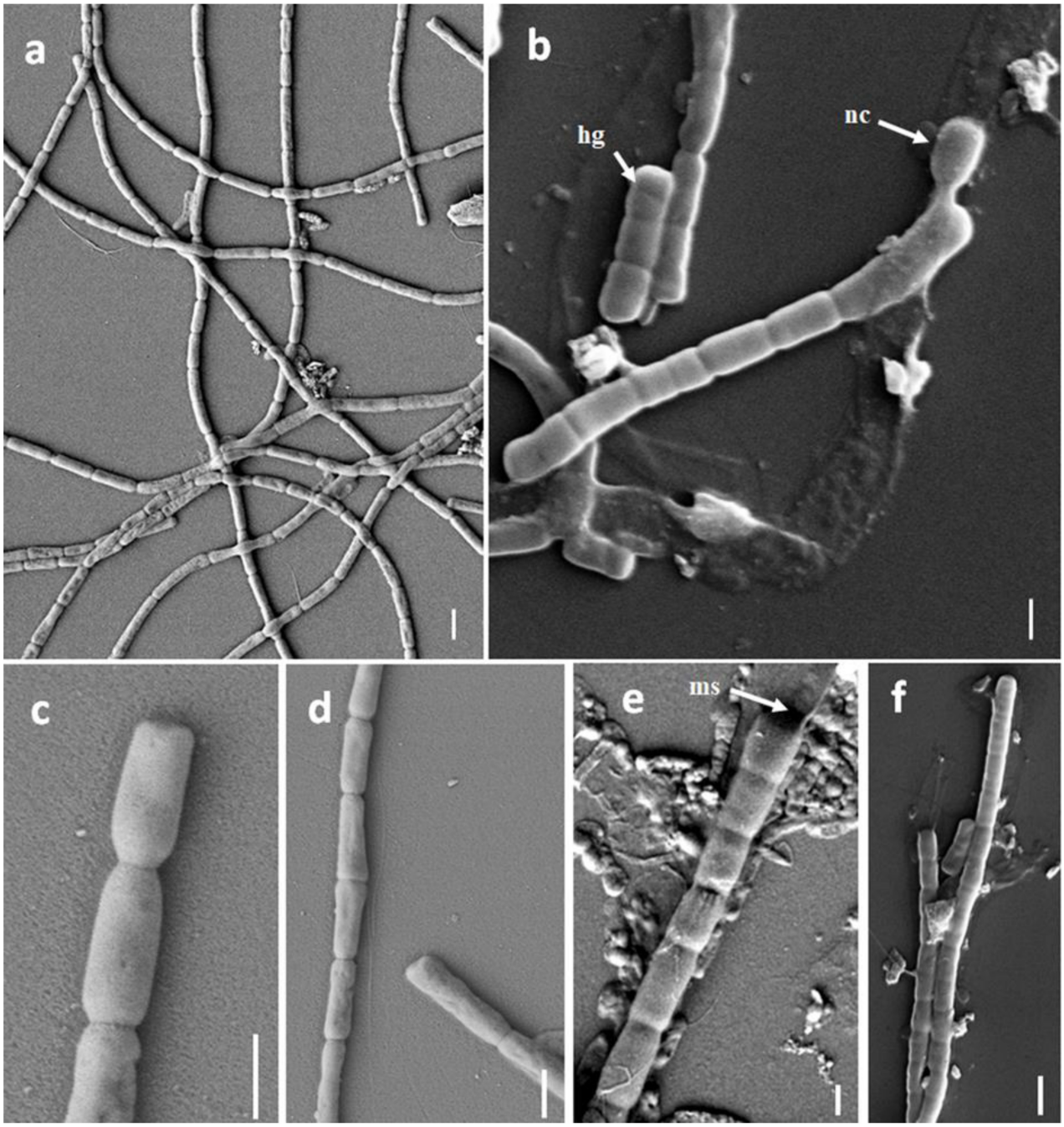

FIGURE 3. Scanning electron microscopy of strains AP3 and AP3b. **a, c** and **d.** Filaments of strain AP3. **b.** Filaments of strain AP3b (non-axenic) showing a hormogonium and a necridic cell. **e** and **f.** Filaments of strain AP3b showing presence of a facultative mucilaginous sheath. hg = hormogonium; nc = necridic cell; ms = mucilaginous sheath

TABLE 2. Comparative analysis of the morphological features of test strain *Euryhalinema pallustris* AP3 and reference strain *Euryhalinema mangrovii*. Characteristics of previously described genus *Euryhalinema mangrovii* AP9F was obtained from Chakraborty *et al.* (2019).

Feature	***Euryhalinema pallustris* AP3**	***Euryhaliema mangrovii* AP9F**
Thallus	Thin biofilm of light greenish color	Pale bluish-green color mats
Filaments	Thin, long, unbranched, isopolar, straight	Thin, long, unbranched, straight, isopolar
Mucilage sheath	Absent	Absent
Cell length (μm)	1.1 – 1.6	1.25 – 2.6
Cell width (μm)	0.4 – 0.5	0.4 – 0.6
False branching	Absent	Absent
Cell form	Distinctly much longer than wide	Distinctly much longer than wide
Cross walls	Constricted	Constricted
Trichome apex	Rounded, not attenuated	Rounded not attenuated
Habitat	Sub-aerophytic, in the soils of intertidal area of estuary with salinity ranging from 1.7-1.8 %	Sub-aerophytic, in the soils of intertidal area of estuary with salinity ranging from 1.7-1.8 %

TABLE 3. Comparison of the features of *Aerofilum faciculatum* AP3b along with other related genera of the family Oculatellaceae. Features of previously described genera were extracted from Osorio-Santos *et al*. (2014) (for *Oculatella atacamensis*) and Mai *et al.* (2018) (for other published genera of Oculatellaceae).

	Aerofilum fasciculatum	*Oculatella atacamensis*	*Pegethrix convoluta*	*Drouetiella hepatica*	*Timaviella radians*	*Tildeniella torsiva*
Thallus	Blue-green, trichomes appears in clusters like 'fascicles'	Thin mat, growing diffusely from the centre	Bright blue-green, radially spreading in the agar	Brownish or purplish-brown floating mucilaginous mats	Thallus leathery, dark olive-brown to olive-green colour	Bright blue-green colour colony spreading irregularly, fasciculated
Filaments	Filaments long, straight, isopolar	Filaments flexuous, long, straight	Filaments long, fasciculated, sometimes form nodules, straight or bent	Filaments long, straight	Filaments relatively short, wide, forming radial colonies	Filaments flexuous or spirally coiled
Cell length (μm)	1.4 - 2.1	1.5 - 7.4	1.0 - 2.5	3.1 - 4.5	1.2 - 2.2	1.5 - 2.7
Cell width (μm)	0.9 - 1.1	1.5 - 2.3	1.3 - 2.5	2.8 - 3.7	1.8 - 3.7	1.7 - 2.5
Cell form	Cells slightly longer than wide to isodiametric	Cells mostly longer than wide	Cells shorter than wide but after division longer than wide	Cells mostly longer than wide to elongated with a central at centre	Cells shorter than their width	Mostly longer than wide, rarely isodiametric
Cross walls	Distinctly constricted	Slightly constricted with oblique wall formation	Slightly constricted	Slightly constricted	Distinctly constricted	Slightly constricted
Sheath	A thin facultative sheath present	Distinct sheath present	Distinct sheath present	Distinct, firm sheath present	Sheath diffluent, rarely distinct	Distinct, firm, thin sheath
False branching	Absent	Rarely present	Infrequently present	Occasionally present	Present	Rarely present
Apical cell	Rounded end apical cell with homogeneous contents	Bluntly-rounded end apical cell with reddish-orange spot in the apex	Rounded apical cell	End cells with cylindrical-rounded end	Round to conical round ended apical cell	Rounded end apical cells
Aerotopes	Present	Absent	Absent	Absent	Absent	Absent
Necridic cells	Present	Absent	Present	Present	Absent	Absent
Habitat	Sub-aerophytic soil biofilm from intertidal estuarine region	Rock-soil interface in Atacama Desert	Soil microbial layer from a seep wall in USENM, USA	Sub-aerophytic, found in limestone. Slovakia	Mats in the waterfall, Navajo Sandstone, USA	Sub-aerophytic mats on the limestone wall, Slovakia

3.2 Ultrastructural studies Transmission electron microscopic studies on the two strains under investigation were conducted. The microphotographs of the sections of strains AP3 and AP3b revealed the pattern of thylakoidal arrangement as well as the granulation which were in agreement with the archetype of the thylakoids of the family Leptolyngbyaceae (Komarek & Anagnostidis 2005) as well as with the family Oculatellaceae (Mai *et al.* 2018). The thylakoidal arrangement of strain AP3b was peripheral. The presence of facultative sheath in the cross section was also confirmed by transmission electron microscopy (Fig. 4d). The cross section of the cell also disclosed the presence of aerotopes which were colorless, nearly circular in shape, irregularly distributed throughout the cell and were of various sizes, 4-7 in numbers per cell (Fig. 4c). Strain AP3 showed the presence of various granulations like carboxysomes and polyphosphate bodies (Fig. 4b) in the cross section of its cells.

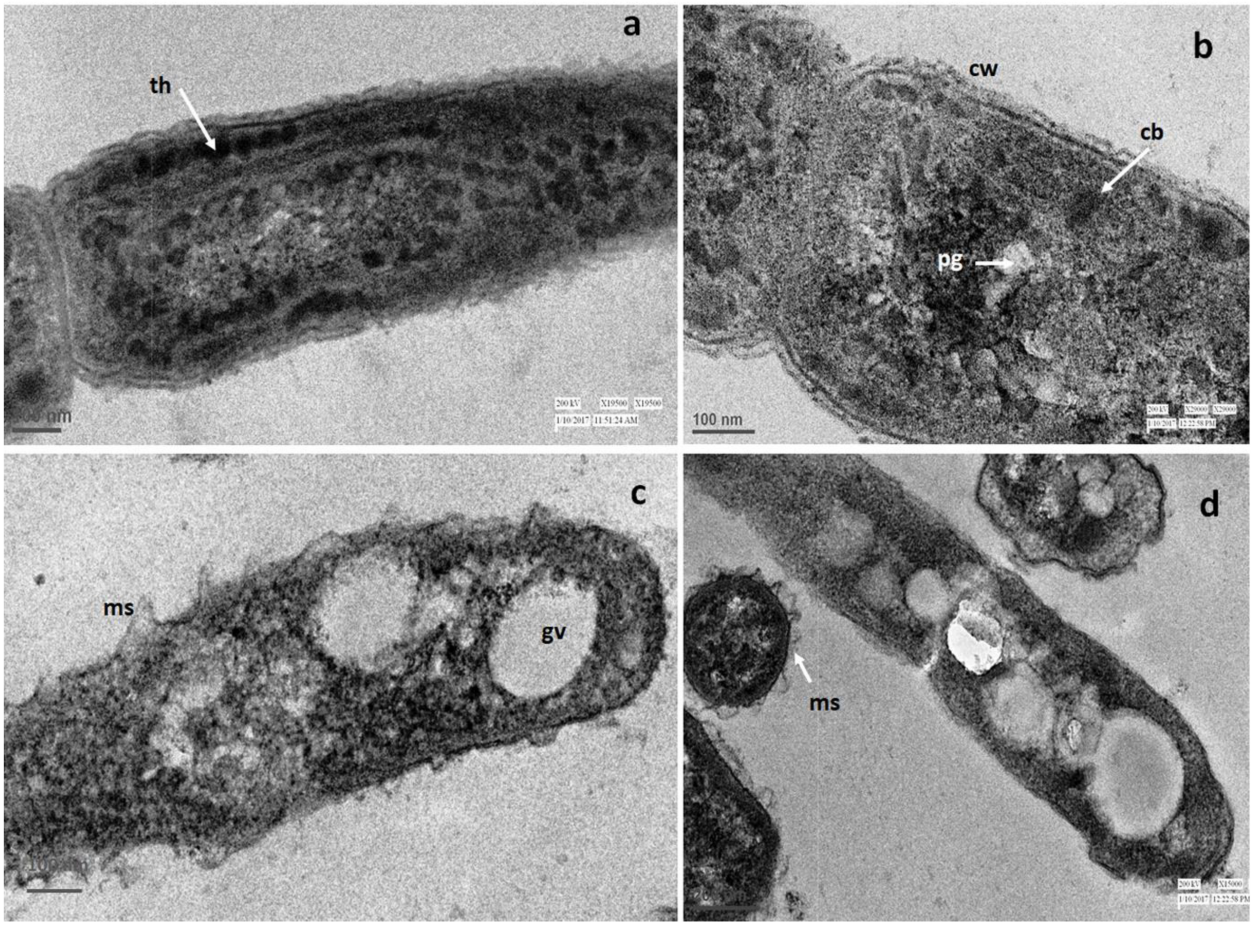

FIGURE 4. Transmission electron microscopy of strain AP3 and AP3b. **a** and **b.** Cross-section of a part of filament of strain AP3 showing thylakoidal arrangement and granulations. **c** and **d.** Cross-sectional view of cell of strain AP3b showing aerotopes distributed throughout the cell. th = thylakoids; pg = polyphosphate granules; cb = carboxysomes; cw = cell wall; ms = mucilaginous sheath; gv = gas vesicles.

3.3 Alignment of the 16S rRNA gene sequence and phylogenetic analysis A partial sequence of the 16S rRNA gene of AP3 (1282 nucleotides) and AP3b (1126 nucleotides) was obtained by the PCR as described in section 2.5. The sequences obtained for both the strains were checked and authenticated with appropriate quality control values as well as verified to be pure without any chimera. The two sequences were compared with the other sequences available in GenBank. The nearest hit showing highest similarity (about 98.2%) with strain AP3 was *Euryhalinema mangrovii* strain AP9F (Accession number MK402979) which was recently published as a monospecific genus by us (Chakraborty *et al.* 2019). The second hit was with the cyanobacterium named '*Calothrix* sp. 96/26 LPP3' (Accession number KM019977) having similarity around 97%. This strain was incorrectly named and submitted in the GenBank and should be revised for a proper taxonomic affiliation (Chakraborty *et al.* 2019). Barring these two cyanobacteria, others strains in the NCBI hits were found to be significantly distant from our isolate AP3 having similarities less than 94%. Hence, it was decided to select *Euryhalinema mangrovii* as the reference strain for the test strain AP3 for further phylogenetic analysis and eventual proposal as a novel species under the genus *Euryhalinema* in this present communication. Furthermore, the strain AP3 established itself as a sister taxon with the genus *Euryhalinema* and

along with '*Calothrix* sp. 96/26 LPP3' formed a well-supported clade sister to the clade of genus *Leptoelongatus litoralis*. *L. litoralis* was concomitantly a novel genus described by us (Chakraborty *et al.* 2019) from the same field area of the Indian Sundarbans. The phylogenetic tree consisted of the members of Synechococcalean order including some of the genera of family Leptolyngbyaceae, almost all the genera of family Oculatellaceae (Mai *et al.* 2018) and key genera from the family Prochlorotrichaceae as proposed by Becerra-Absalon *et al.* (2018).

The closest relative of strain AP3b in the NCBI was a strain named *Oculatella atacamensis* (Accession number KF761587) which demonstrated about 93.82% genetic similarity. The second top hit with proper designation mainly included the species of genus *Tildeniella* (KY498228) of the family Oculatellaceae with a similarity about 92.77%. Other hits also included genera of Oculatellacean members like *Timaviella radians* (KY078774) and *Drouetiella hepatica* (HM018689), displaying a range of 92-90% genetic similarity with the test strain AP3b. This similarity (as percentage) was definitely justified in context to the threshold likeliness in between two genera, which should be less than 94.7% (Yarza *et al.* 2014). Therefore, the morphological likeliness as well as the molecular similarity leads to the proposal that strain AP3b must belong to the family Oculatellaceae. Species of the genus *Oculatella*, i.e., *Oculatella subterranea* (Zammit *et al.* 2012) and *O. atacamensis* (Osorio-Santos *et al.* 2014) along with other genera of family Oculatellaceae were selected as the reference strains solely on the basis of the genetic similarity of 16S rRNA gene sequence for further phylogenetic studies. Subsequently, a consensus phylogenetic tree was constructed including all the clones of the strains under investigation (AP3 and AP3b) along with their closest relatives and other members of the family Oculatellaceae and Prochlorotrichaceae to justify the evolutionary relationship. The outcome of the tree demonstrated that the clones of strain AP3b were clustered together in a well-supported clade under the family clade of Oculatellaceae and separated from its nearest relative *Oculatella* genus and other genus clades and formed a novel distant phylogenetic lineage according to BI and ML analyses supported by high posterior probability and bootstrap values (Fig. 5). As the resulting trees from the ML and BI method provided closely matching topologies, only the ML tree is presented in Fig. 5 with the bootstrap values for node support along with the Bayesian posterior probabilities (Chakraborty *et al.* 2018, Chakraborty *et al.* 2019). The expanded version of the phylogenetic tree can be viewed in Supplementary Figure S1.

The analysis of p-distance for establishing an inter-species relationship between AP3 and *Euryhalinema mangrovii* AP9F showed 98.3% similarity for 16S rRNA data (Table 4) and a dissimilarity of their ITS regions about 10.6% among both the strains (Table 5). The p-distance analysis for the sequences compared for AP3b revealed that the similarity (as percentage) varied in the range 89% to 93% similarity (Table 6) among the members of family Oculatellaceae including strain AP3b while the dissimilarity percentage for their ITS sequences displayed a range from 15.9% to 30.7% (Table 7). The various conserved helices of 16S rRNA were identified and folded for both the strains under investigation. The folded conserved structures for the strains were represented in Supplementary Figure S2. The aligned sequences of the conserved helices of 16S rRNA were also displayed as Supplementary Table S3.

TABLE 4. Similarity (as percentage) of strain AP3 along with *Euryhalinema mangrovii* AP9F based on p-distance analysis of 16S rRNA gene sequence data. Isolate studied in this investigation indicated in bold font.

		1	2	3
1	***Euryhalinema pallustris* AP3 clone 1**			
2	***Euryhalinema pallustris* AP3 clone 2**	99.9		
3	***Euryhalinema pallustris* AP3 clone 3**	100	99.9	
4	*Euryhalinema mangrovii* AP9F	98.3	98.3	98.3

TABLE 5. Dissimilarity (as percentage) of strain AP3 and *Eyryhalinema mangrovii* based on p-distance analysis of 16S-23S ITS gene sequence data. Isolate investigated in this manuscript indicated in bold font. Only the operon with both tRNA genes were considered for each strain under comparison.

		1	2	3
1	***Euryhalinema pallustris* AP3 clone 1**			
2	***Euryhalinema pallustris* AP3 clone 2**	0.00		
3	***Euryhalinema pallustris* AP3 clone 3**	0.20	0.20	
4	*Euryhalinema mangrovii* AP9F	10.6	10.6	10.8

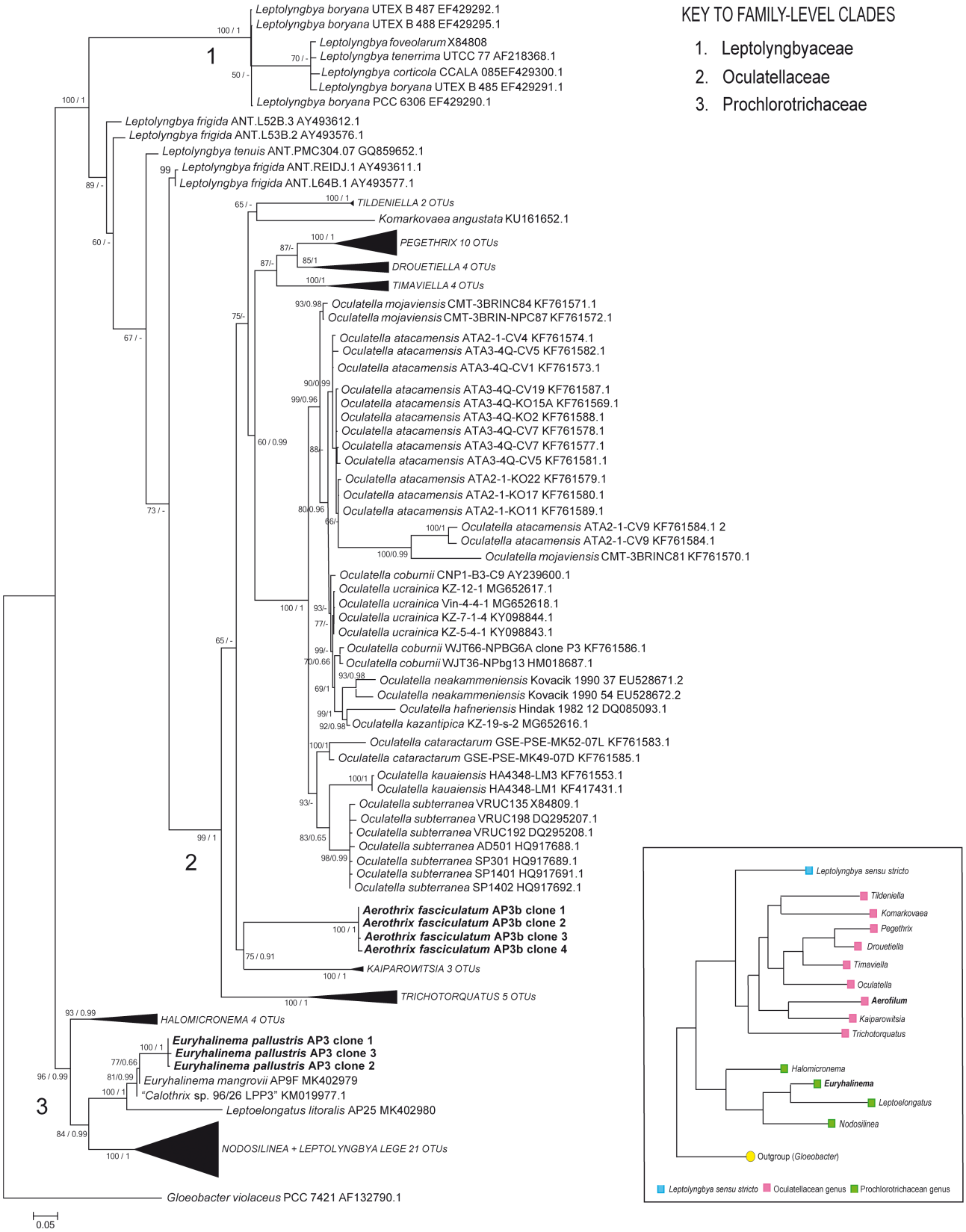

FIGURE 5. Phylogenetic tree based on 16S rRNA gene sequences of total 115 OTUs belonging to 3 families of Synechococcalean order and *Gloeobacter violaceus* as outgroup. Bootstrapping with 1000 resamplings was performed. Support values are ML bootstrap/ BI posterior probability. Scores denoted by '-' for any node showed no support in that analysis. The investigated strains with clones (AP3 and AP3b) are shown in bold. Taxon name in quotation mark, e.g. "*Calothrix"* in our opinion represents an incorrectly submitted sequence and requires revision. Collapsed clades can be viewed expanded in Fig. S1. Designation of families according to Mai *et al.* (2018).

TABLE 6. Similarity (as percentage) of strain AP3b and some strains of family Oculatellaceae based on p-distance analysis of 16S rRNA gene sequence data. Isolate studied in this investigation indicated in bold font.

		1	2	3	4	5	6
1	***Aerofilum fasciculatum* AP3b**						
2	*Oculatella subterranea* VRUC192	92.8					
3	*Oculatella atacamensis* ATA2-1-CV24	92.9	97.2				
4	*Pegethrix convoluta* GSE-PSE-MK38-07D	89.0	92.7	92.1			
5	*Drouetiella hepatica* UHER 2000/2453	89.5	92.9	93.2	93.6		
6	*Timaviella radians* GSE-TBD6-7R	90.3	93.1	93.1	91.3	92.7	
7	*Tildeniella torsiva* Lubos34 UHER 1998/13d	92.5	92.7	93.1	91.4	92.1	93.6

TABLE 7. Dissimilarity (as percentage) of strain AP3b and some strains of family Oculatellaceae based on p-distance analysis of 16S-23S ITS gene sequence data. Isolate studied in this investigation indicated in bold font. Only the operon with both tRNA genes were considered for each strain under comparison.

		1	2	3	4	5
1	***Aerofilum fasciculatum* AP3b**					
2	*Oculatella atacamensis* ATA2-1-CV24	15.9				
3	*Pegethrix convoluta* GSE-PSE-MK38-07D	22.1	18.4			
4	*Drouetiella hepatica* UHER 2000/2453	24.2	18.2	19.9		
5	*Timaviella radians* GSE-TBD6-7R	30.7	22.0	21.2	20.6	
6	*Tildeniella torsiva* Lubos34 UHER 1998/13d	23.4	17.9	21.8	22.8	23.8

3.4 Analysis of 16S-23S ITS secondary structures The 16S-23S ITS sequence of strain AP3 (475 bp) showed 87.92% similarity with its closest species, *Euryhalinema mangrovii* while the sequence of the strain AP3b (562 bp) showed 83.91% similarity with the nearest relative *Oculatella atacamensis* (KF761575). The complete ITS region of the strains under investigation (AP3 and AP3b) consisting of distinct variable and conserved domains were compared with their respective reference strains to find out the molecular unlikeliness as well as resemblances. This comparison is presented in Table 8. Additionally, the structures of D1-D1' helix, Box-B helix, V2 helix and the V3 region were folded and characterized for the strain AP3b and D1-D1' helix, Box-B helix and V2 helix for AP3. The examined strains possessed two definite operons, one having both the genes for $tRNA^{ile}$ and $tRNA^{ala}$ while the other operon lacked both the genes.

TABLE 8. Comparison of the nucleotide lengths of the ITS regions of *Euryhalinema pallustris* AP3 with *Euryhalinema mangrovii* and *Aerofilum fasciculatum* AP3b with other related genera of family Oculatellaceae. Only operons containing both tRNA genes are reported in this table for each strain. Data of reference strains *Euryhalinema mangrovii* and Oculatellacean genera were obtained from Chakraborty *et al.* (2019), Osorio-Santos *et al.* (2014) and Mai *et al.* (2018).

Strain ID	Leader	D1-D1' helix	Spacer + D2 + spacer	D3 + spacer	$tRNA^{ile}$ gene	V2 spacer	$tRNA^{ala}$ gene	Pre-Box B spacer	Box B	Post Box B spacer	Box A	D4	V3	D5
Euryhalinema pallustris AP3	8	63	39	9	74	15	73	37	32	24	11	11	24	18
Euryhalinema mangrovii AP9F	8	63	36	12	74	7	73	34	32	19	11	7	20	16
Aerofilum fasciculatum AP3b	7	73	41	13	74	12	73	56	38	15	11	22	49	17
Oculatella atacamensis ATA2-1-CV24	7	62	37	11	74	8	73	33	33	15	11	23	52	14
Pegethrix convoluta GSE-PSE-MK38-07D	7	91	12	33	74	14	73	64	36	19	11	16	110	23
Drouetiella hepatica UHER 2000/2452	7	64	35	12	74	42	73	39	34	19	11	14	52	51
Timaviella radians GSE-TBD6-7R	8	81	42	22	74	14	73	41	33	18	11	14	59	34
Tildeniella torsiva UHER 1998/13D	7	66	33	14	74	11	73	35	49	18	11	15	92	16

The conserved basal sequences of strain AP3 were identified as carried out for *Euryhalinema mangrovii* (Chakraborty *et al.* 2019). The D1-D1' helix of strain AP3 (65 nt) was characterized by the presence of one short terminal loop (5 nt) followed by a large single bilateral bulge and two small bilateral loops. A unilateral bulge (7 nt) near the basal stem region was present which was a conserved structure of the D1-D1' helix in most of the Synechococcalean members; however, the sequences varied from species to species. On the contrary, the D1-D1' helix of the reference strain

Euryhalinema mangrovii consisted of 63 nucleotides and the structure depicted the overall pattern to be similar to the D1-D1' helix of AP3 but there existed variations in sequences due to substitution of nucleotides (Fig. 6). On the other hand, the complete structure of Box B helix for both AP3 and *Euryhalinema mangrovii* AP9F were almost similar with a length of 32 nt consisting of a terminal loop (6 nt) and a single nucleotide bulge near stem region. Nucleotide substitution occurred in strain AP3 in two consecutive bases in the terminal loop and a non-canonical base pairing 5'-G::U-3' substituted a canonical base pairing 5'-A::U-3'. The secondary structure of the V2 helix in case of strain AP3 contained 15 nucleotides with a 5 nt terminal loop and a basal stem. This structure differed from the V2 region of *Euryhalinema mangrovii* having a very small helix of length 7 nt.

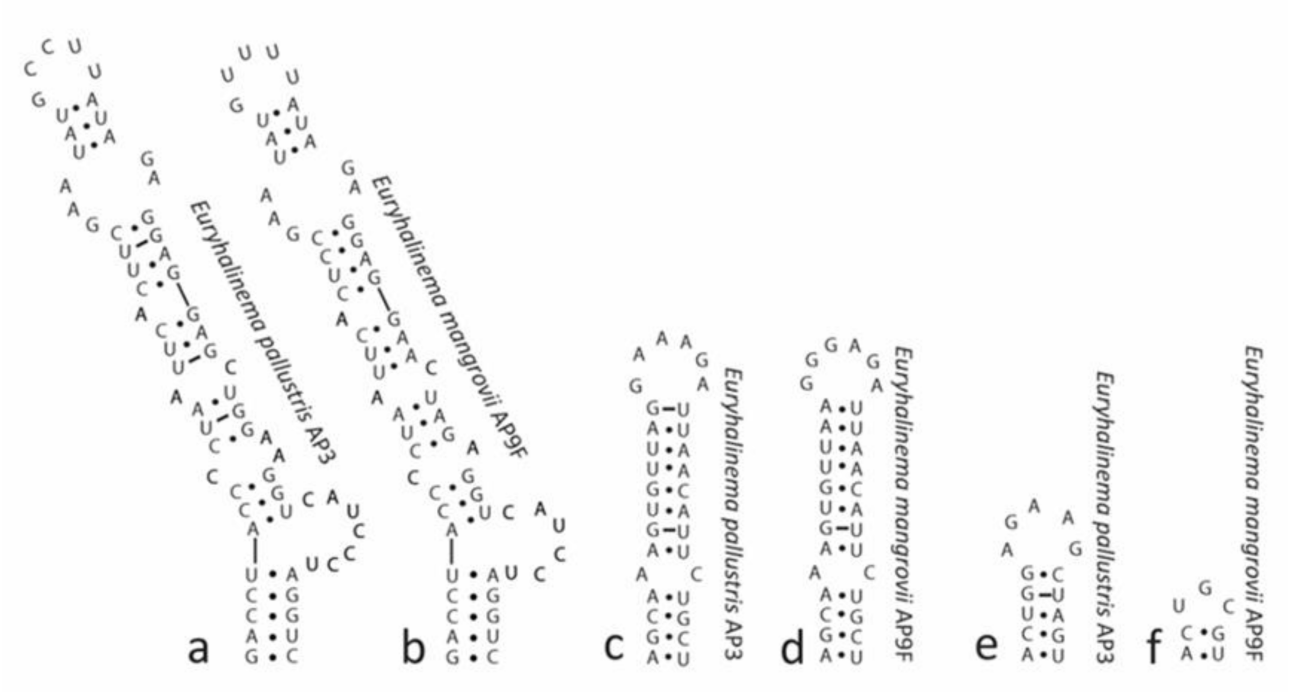

FIGURE 6. Comparative analysis of D1-D1' helix, Box-B and V2 helix of 16S-23S ITS region of test strain AP3 (*Euryhalinema pallustris*) and reference strain *Euryhalinema mangrovii*. **a-b.** D1-D1' helix. **c-d.** Box-B helix. **e-f.** V2 helix

Analysis of the ITS folded secondary structures of strain AP3b in comparison to other strains of family Oculatellaceae revealed that strain AP3b contained unique genus-specific features in the ITS structures. The D1-D1' helix for strain AP3b was 73 nt long, constituting of a small terminal loop (5 nt) followed by a large bilateral bulge and a small, 2 nt bulge in the middle of the helix. A unilateral bulge near the basal stem existed which possessed a unique sequence of nucleotides which differed from the other genera under comparison (Fig. 7). All the strains possessed the same basal sequence 5'GACC::CUGG3' in the D1-D1' helix. Box-B helix domain of the ITS secondary structure of strain AP3b was 38 nucleotides long, with two small sized bilateral bulges and a terminal loop (5 nt) while the corresponding Box-B structure for other genera under comparison varied in length and differed in the structural features (Fig. 7). The comparison of V2 and V3 helices of AP3b along with other Oculatellacean members (Fig. 8) displayed many variations among them. V2 helix and V3 helix of strain AP3b were 12 nt and 49 nt long respectively. V2 helix possessed a terminal loop (4 nt) and a small basal stem. V3 helix of AP3b showed a terminal loop (4 nt), two large bilateral bulges in the middle of the helix and a small, 2 nt unilateral bulge in the lower portion of the helix. Fig. 8 depicts an overall comparison of V2 and V3 helices of AP3b with other Oculatellacean members disclosing that these helices varied in length as well as sequence when compared among each other.

Discussion

This article describes the study and taxonomic characterization of two cyanobacterial strains using the polyphasic approach to taxonomy. This study is a sequel to the investigation of Pramanik *et al.* (2011) who isolated eight cyanobacterial strains from the Sagar and Lothian islands of Indian Sundarbans and primarily assigned them to the LPP (*Lyngbya-Phormidium-Plectonema*) Group B and Oscillatoriales groups. Further analysis on four of the eight

cyanobacteria performed by Chakraborty *et al.* (2018) and Chakraborty *et al.* (2019) following the polyphasic approach to taxonomic analyses established *Oxynema aestuarii sp. nov.* (Microcoleaceae) as a novel species and *Euryhalinema mangrovii gen. nov., sp. nov.* as well as *Leptoelongatus litoralis gen. nov., sp. nov.* (Leptolyngbyaceae) as novel genera. These two novel genera were described by Chakraborty *et al.* (2019) under the family Leptolyngbyaceae by following Komarek *et al.* (2014) although Becerra-Absalon *et al.* (2018) defined the same family clade as Prochlorotrichaceae. Two other strains (namely AP3 and AP3b) from the collection of Pramanik *et al.* (2011) were compared with close members of the *Euryhalinema* and Oculatellacean genera on the basis of the polyphasic approach to taxonomic analyses incorporating molecular phylogenetic relationships. Many phenotypic features overlapped between the investigated strains and the reference strains due to their simple morphology. However, molecular phylogenetic analyses along with ecological considerations and some distinct cellular features (presence of aerotopes, fascicular growth pattern in case of AP3b) advocated that the strains AP3 and AP3b should be designated as novel species and genus respectively. The discussion of this work has been suitably divided into two sections, each of which includes the justification of the proposed taxonomic assignment of the two isolates investigated in this study, namely AP3 and AP3b.

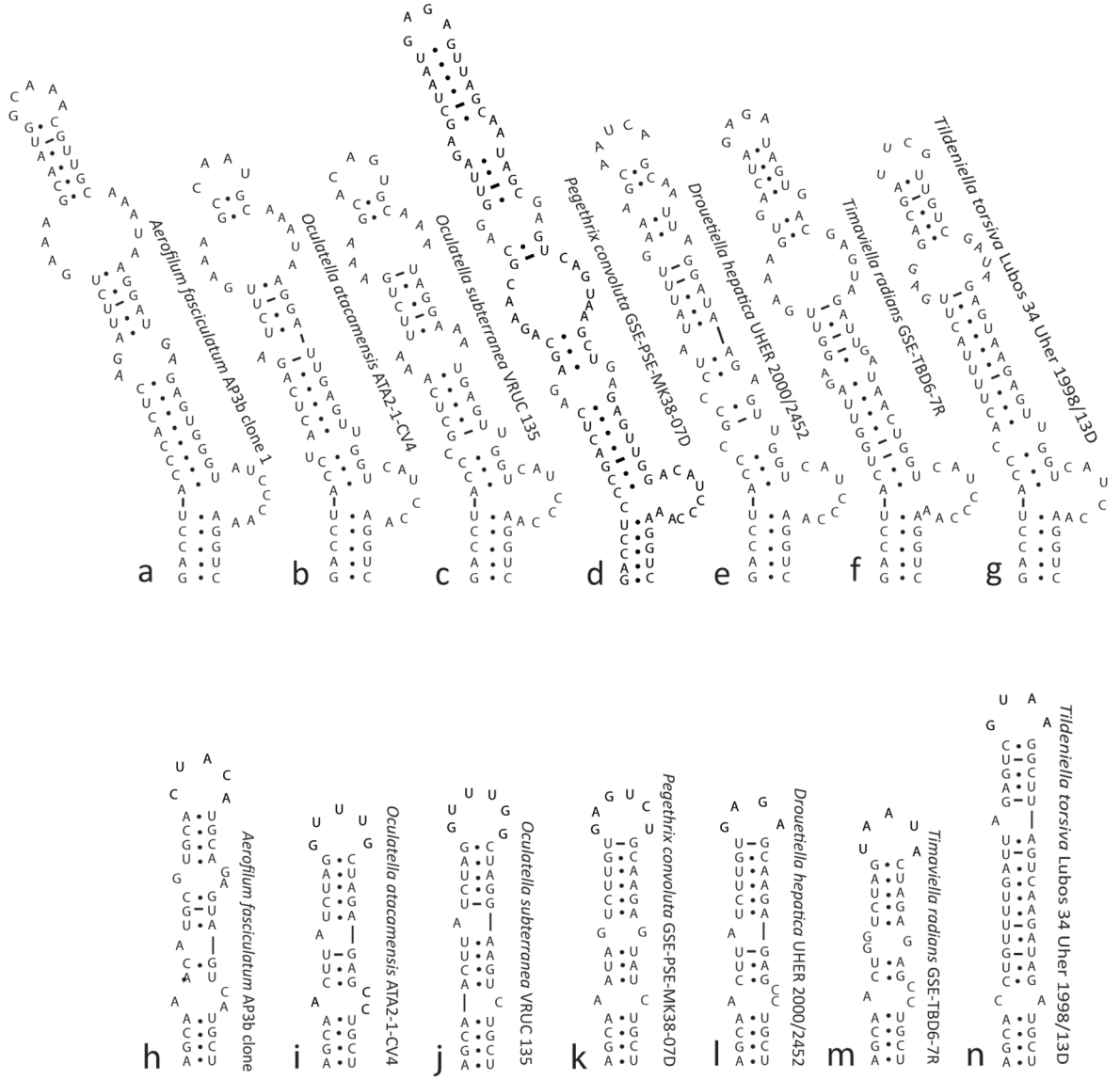

FIGURE 7. Comparative analysis of D1-D1' helix and Box-B helix of 16S-23S ITS region of test strain AP3b (*Aerofilum fasciculatum*) with the genera of Oculatellaceae family. **a-g.** D1-D1' helix. **h-n.** Box-B helix.

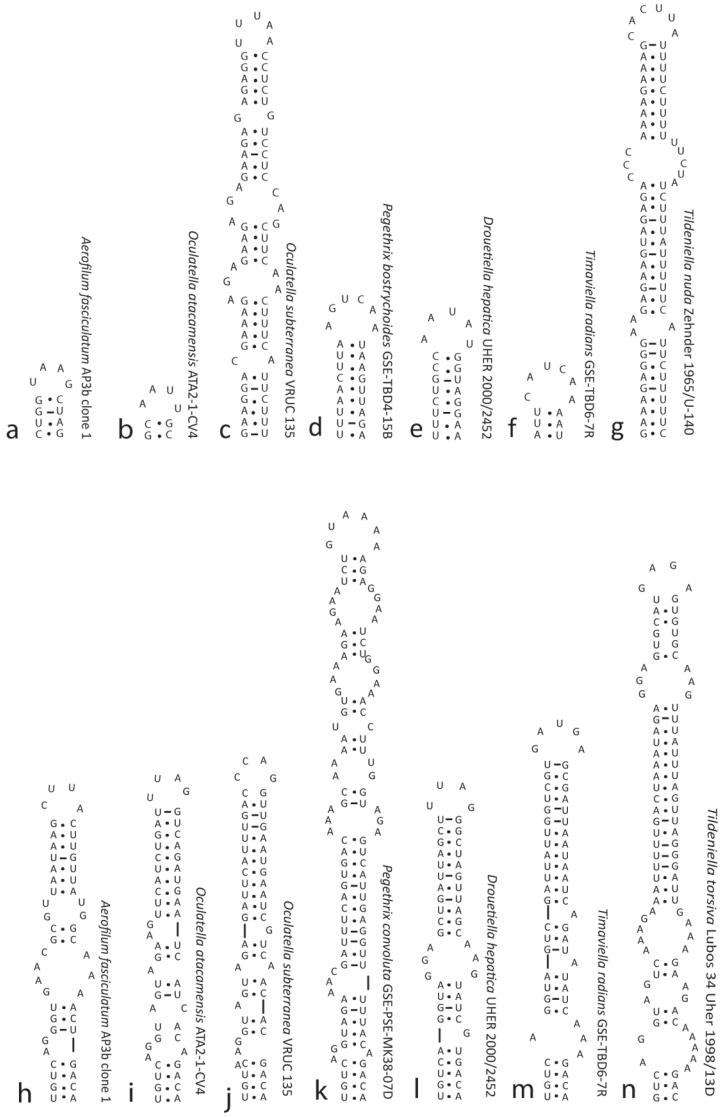

FIGURE 8. Comparative analysis of V2 and V3 helix of 16S-23S ITS region of test strain AP3b (*Aerofilum fasciculatum*) with the genera of Oculatellaceae family. **a-g.** V2 helix. **h-n.** V3 helix.

We provided evidences based on the polyphasic approach to taxonomy to claim that isolate AP3 should be considered as a novel species under the genus *Euryhalinema*. Following the cladistical information provided by Mai *et al.* (2018), the strain AP3 along with its proposed sister species AP9F (Chakraborty *et al.* 2019) fits well in the family Prochlorotrichaceae. This was also supported by the analysis of the conserved helices of 16S rRNA performed in this article (Supplementary Table. S3). Comparison of helix 23 and 27 of AP3 with the sequences for different within Synechococcales also showed affinity of AP3 with Prochlotrichaceae. This analysis was an important observation for family affiliation (Mai *et al.* 2018). In general, morphological data were corroborative with the phylogenetic studies (Chakraborty *et al.* 2019). The comparison of strain AP3 with its reference strains was primarily focused on the molecular data. The sequence similarity of the 16S rRNA gene sequence was found to be around 98.2% which was comparable to the recommended value to ascertain inter-specific differentiation (Yarza *et al.* 2014). In a recent work reported by Jung *et al.* (2020), delineation of a novel species, *Oculatella crustae-formantes* from *O. ucrainica* was based on 98.8% genetic similarity of 16S rRNA sequence. Similarly, other reports primarily, (Osorio-Santos *et al.* 2014, Vinogradova *et al.* 2017, Mai *et al.* 2018) also followed the same threshold of less than 98.7% genetic similarity set by Yarza *et al.* (2014) for species delineation. Hence, in this present study delineation of two species under a single genus (*Euryhalinema*) was primarily well supported by molecular evidence also combined with morphological

and ecological confirmations. Phylogenetic tree (Fig. 5) also demonstrated well-supported clade to validate strain AP3 as the second and a novel species under the genus *Euryhalinema*. The inclusion of a wrongly named strain "*Calothrix* sp. 96/26 LPP3" into the tree also indicated that it can further be established as a novel species under this clade following the polyphasic approach to taxonomic analysis (Chakraborty *et al.* 2019). Molecular analysis for the delineation of species also involved the study of 16S-23S ITS secondary structures which was considered to be an essential evaluation criterion for the alpha-level taxonomy (Boyer *et al.* 2001, Johansen *et al.* 2011, Chakraborty *et al*. 2018). The output of the folded structures of D1-D1' region, Box-B helix and V2 helix region revealed that the overall structure of Box-B helix was slightly different in the investigated strain AP3 when compared with *Euryhalinema mangrovii*; however the structure of D1-D1' and V2 helices differed among the test and reference strains (Fig. 6). The D1-D1' helix comparison revealed nucleotide substitutions in five different positions in the strain AP3, while the basic conserved motifs remained alike. These motifs were always conserved for the post-transcriptional processing of the ribosomal operon (Johansen *et al.* 2011). The unilateral bulge near the basal stem showed a mutation which resulted in a different sequence in AP3 (5'-CAUCCCU-3') in comparison to the *Euryhalinema mangrovii* (5'-CAUCCU-3'). The terminal loop also contains a 2-nucleotide substitution as in AP3 (5'-GCC-3') which differed from the reference (5'-GUU-3'). The stem region also contained minor substitutions which implied the sequential insertion-deletion events articulating the evolutionary changes during speciation (Johansen *et al.* 2011). V2 region of AP3 was significantly longer than the V2 region in *Euryhalinema mangrovii* (Fig.6) with varying terminal loops among them. However, only the structure of Box-B showed differences among their terminal loops with a substitution of 5'-GAA-3' in AP3 to 5'-GGG-3' in the reference strain. According to Johansen *et al.* (2011), the conserved and variable regions of the ITS sequences were not flexible like any morphological features. Moreover, due to strong selection pressure the changes of the ITS regions were much more stable and hence reliable for species identity. The separation of a novel species *Leptolyngbya corticola* from another species of the *Leptolyngbya* genus on the basis of the ITS folding patterns was described by Johansen *et al.* (2011). Recent works in the taxonomical revisions of cyanobacteria also included the comparative analysis of dissimilarity (as percentage) of the ITS regions based on the p-distance analysis, especially to establish an inter-species relationship (Erwin and Thacker 2008, Osorio-Santos *et al*. 2014, Pietrasiak *et al*. 2014, Johansen *et al.* 2017, Shalygin *et al.* 2017, Gonzalez-Resendiz *et al.* 2018a,b, Mai *et al*. 2018, Vazquez-Martinez *et al.* 2018) with a threshold value of >7% dissimilarity to be considered as separate species (Gonzalez-Resendiz *et al.* 2019). The dissimilarity (as percentage) of ITS for AP3 and *Euryhalinema mangrovii* was observed to be 10.6% (Table 5) which reflected their status to be two different species under the genus *Euryhalinema.* Gonzalez-Resendiz *et al.* (2019) showed that although the ITS secondary structures among the species do not show significant differences, still the separation of *Desertifilum fontinale* from the other species, *D. tharense, D. dzianense* and *D. salkalinema* was reported mainly on the basis of >3% dissimilarity in ITS regions by p-distance analysis.

Morphological features of AP3 like absence of sheath, type of constriction in the cross walls and apical cell morphology were considered as synapomorphies that were in common with the species *Euryhalinema mangrovii* which further corroborated its assignment as a novel species under the same genus (*Euryhalinema*). Only the cell size differed between AP3 and the reference strain which could be attributed to the variation in their habitats. The morphological changes due to long-term culturing was not detectable, and therefore the difference in the cellular length could be considered as a diagnosable feature in this case. This evidence was supported by the findings of Zhou *et al*. (2018), who described two novel genera based on the differences in cellular length which was considered as an autapomorphic feature. Besides the genetic isolation, the ecological origin of both AP3 and *Euryhalinema mangrovii* also suggested that the two isolates must have evolved as two different species under the genus *Euryhalinema* because the two species were collected from two different islands of the Indian *Sundarbans* separated by 24 kms (Fig. 1). Thus, AP3 and *Euryhalinema mangrovii* are two ecotypes isolated geographically.

The second strain investigated in this study was AP3b which was very interestingly proposed to be a new genus under the recently described novel family Oculatellaceae (Mai *et al.* 2018). The affiliation to family Oculatellaceae of the strain AP3b was primarily supported by 100% similarity of helix 23 and 27 with family Oculatellaceae (Supplementary Table S3). This claim is very well supported by various morphological, ultrastructural and molecular data examined in the present work. Strain AP3b was found to be more than 6.2% variable (in terms of genetic variability compared with other genera) based on the 16S rRNA sequence data (Table 6) with the genus *Oculatella atacamensis*, *Tildeniella torsiva* and other Oculatellacean members. All the clones of strain AP3bclustered together and were well separated as a lineage from the *Oculatella* and the *Tildeniella* genus clade. Becerra-Absalon *et al.* (2018) had separated the novel genus *Chroakolemma* sp. from its closest relative *Scytolyngbya timoleontis* placed as the nearest clade in the phylogenetic tree on the basis of more than 5% genetic variability. Additionally, Mai *et al.* (2018) described six new genera containing 14 new species under the novel family Oculatellaceae where genetic variation among the genera

belonging to same families was on average 6.7 – 8.5%. Our strain AP3b also belonged to the clade next to the genus-level clade of *Kaiparowitsia* and *Oculatella,* well-fitted phylogenetically under the family Oculatellaceae along with the other Oculatellalean genera. Based on this evolutionary relationship of the strain AP3b with other genera in the phylogenetic tree, we considered all the genera of the family Oculatellaceae to be satisfactory representatives for inter-generic comparative analysis with our strain AP3b. Mai *et al.* (2018) further explained that the genera under the family Oculatellaceae revealed many autapomorphic specificities based on the study of the secondary structures of the ITS region. The folded secondary structures of ITS region of strain AP3b also disclosed a substantial degree of dissimilarity in comparison to the other members of Oculatellaceae not only in its conformation but also in its sequence length. The lengths of various regions of ITS of strain AP3b compared in Table 8 revealed that the conserved and variable regions of the members of Oculatellaceae possessed high variation where the length of various regions of ITS of each genus was specific. The D1-D1' helix which was considered to be highly conserved differed significantly between strain AP3b and the reference strains. The basal sequence of the helix (5'GACC-GGUC 3') was common to strain AP3b and all other compared genera (Fig. 7) which appeared to be a confirmed feature of the family Oculatellaceae (Mai *et al.* 2018). The unilateral bulge near the basal stem of D1-D1' helix for all of the strains in comparison was found to be varying in 1-2 nucleotides among the genera. Similarly, in case of AP3b, a unique sequence 5'-AUCCCAA-3' was observed (Fig. 7) which was dissimilar compared to any other genera of the Oculatellaceae family. Moreover, only genus *Pegethrix* (Mai *et al.* 2018) possessed very large structure of the D1-D1' helix (91 nt) and the corresponding structure of AP3b was found to be significantly longer than any other genera except *Pegethrix.* The terminal loop also differed in size as well sequence when compared with the references (Fig. 7). Box-B helix of strain AP3b was also noticed to be considerably longer than the Box-B helix of its closest related member genus *Oculatella*. Besides the sequence variation of the terminal loop, AP3b also differed in possessing an extra small bilateral bulge in comparison to the genus *Oculatella* (*O. atacamensis* and *O. subterranea*). Box-B helix length in the genus *Tildeniella* is very long and rest of the genera possesses comparably similar length of Box B. So, in the complete Box-B helix comparison with other genera (Fig. 7), it is clear that the length of the Box-B helix in AP3b is unique, being of intermediate length. During the comparison of V2 and V3 helices among strain AP3b and other genera (Fig.8), these highly variable helical regions of the ITS showed a genus-specific conformation where the length of both helices (V2 and V3) for each genus were unique. Moreover, as the homology in the sequences of V2 and V3 compared among the genera was very low, the patterns and number of bilateral bulges as well as the sequences of the stem regions showed significant variations. Considering the ITS region to be a genus-specific trait in family Oculatellaceae, the D1-D1' helix, Box-B, V2 and V3 region represented in Fig. 7 and Fig. 8 presented significant divergences from strain *Oculatella atacamensis* as well as other Oculatellacean genera. Alike the present investigation, comparison of ITS secondary structures of *Oculatella subterranea* with the type species of *Leptolyngbya* supported the separation of *Oculatella subterranea* as a monospecific genus from the *Leptolyngbya* genus (Zammit *et al.* 2012). According to Mai *et al.* (2018) Oculatellaceae and Prochlorotrichaceae genera had very divergent characters and distinct autapomorphies which were genus specific. This observation was corroborative with our taxonomic assignment of strain AP3b. There were more than one stable and contrasting features identified in the strain AP3b which can undoubtedly be considered as diacritical markers at the genus level. These features included presence of aerotopes in the cross section of the cell causing buoyancy to float which was substantiated by the unique growth pattern of this strain appearing as a fascicle instead of forming mat-like biofilm. Necridic cells were detected by scanning electron microscopy which were contrastingly absent in the reference strain. The related genus *Oculatella* which was regarded as one of the reference strains for AP3b was characterized by the presence of an orange colored spot in its apical cell which was the most striking and genus-specific feature for genus *Oculatella* (Zammit *et al*. 2018). This feature was completely absent in the strain under investigation (AP3b). Moreover, the cellular features of AP3b like nearly isodiametric shape along with the presence of necridia, aerotopes and distinctly constricted cross walls and the given discontinuity of these features in *Tildeniella* and *Oculatella* were the morphological differences supported by the substantial genetic variability in 16S rRNA sequences. Overall study of strain AP3b demonstrated that this strain consisted of a set of comparable morphological characteristics with respect to the genus-specific characters of the reference genera of Oculatellaceae which was justified by the position of strain AP3b under the family Oculatellaceae. Genus can be defined as a group of one or more species bearing at least some morphological characters which distinguishes them from the species of other genera and must constitute a well-defined monophyletic clade (Mai *et al.* 2018). Although, the simple morphology of strain AP3b was similar to the related genus *Oculatella*, however the studied characters like presence of aerotopes aiding to the fasicular growth pattern, presence of necridia, significant differences in the cell forms (cellular length to width ratio) and absence of any orange-colored spot in the apical cell in any phase of its growth period were substantial and stable features to be considered as autapomorphic characters differentiating a species from another species of different genus. Justifying the definition of

genus (Mai *et al.* 2018), and on the basis of the above-mentioned contrasting morphological features substantiated by the molecular data of unlikeliness with the closest member (sister clade), we propose the novel monospecific genus, *Aerofilum fasciculatum* under the family Oculatellaceae.

Conclusion

In this article AP3 was described as a novel species *Euryhalinema pallustris sp. nov.* (Prochlorotrichaceae) and AP3b as a novel genera *Aerofilum fasciculatum gen. nov., sp. nov.* (Oculatellaceae) based on the polyphasic approach to taxonomy. This work is important as it corroborated the creation of well-supported monophyletic groups in their respective families. This investigation also brings the world's largest tidal mangrove forest, the Sundarbans to the forefront as a repository of novel cyanobacteria. This intertidal region warrants further exploration for the discovery of new cyanophytes because fossil records indicate intertidal regions were locations of cyanobacterial diversification globally (Demoulin *et al*. 2019).

Description

Order: Synechococcales
Family: Oculatellaceae

Aerofilum Chakraborty et Mukherjee, *gen. nov*.

Thallus blue-green, growth like fascicles or bundles. Filaments isopolar, uniseriate, unbranched, cells slightly longer than their width, cell length ranged from 1.4 - 2.1 µm and width 0.9 - 1.1 µm. Cross walls have distinct constrictions.

Type species (designated here): *Aerofilum fasciculatum* Chakraborty et Mukherjee

Etymology: The generic epithet "*Aerofilum"* is derived from *Aero* Greek for 'air' as the strain possesses gas vesicles (aerotopes) and *filum* Greek for 'filament'.

Aerofilum fasciculatum *sp. nov.* Chakraborty et Mukherjee

Description Thallus blue-green, growth pattern appears like fascicles or bundles rather than forming a mat-like biofilm. Filaments isopolar, uniseriate, unbranched, cells slightly longer than their width, cell length ranged from 1.4 - 2.1 µm and width 0.9 - 1.1 µm. Cross walls have distinct constrictions. Trichomes were cylindrical, immotile, no heterocyte or akinete. Small sized filaments known as hormogonia helps in propagation. Necridic cells present. Ultrastructure includes parietal thylakoids, aerotopes present.

Holotype (designated here): Holotype (AP3b) deposited and cryopreserved in the Microbial Culture Collection (MCC), India having accession number MCC 3478.

Type locality: Lothian island (21.39.1N 88.19.37E) of the Indian Sundarbans, India.

Etymology: The specific epithet *'fasciculatum'* reflects the growth pattern of the strain appearing to grow as a cluster or fascicles instead of forming a mat-like biofilm.

Order: Synechococcales
Family: Prochlorotrichaceae
Genus: *Euryhalinema*

Euryhalinema pallustris Chakraborty & Mukherjee *sp. nov.*

Description Thallus greenish in color, forming a mat-like biofilm. Filaments typically unbranched, straight, isopolar, attached to the soil surface (mainly sub-aerophytic) growing as an extensive mat-like biofilm. Cells of the intermediate trichome were larger in size than the cells towards apex, cells much longer than wide, cellular dimensions 1.1 - 1.6

μm (length) and 0.4 - 0.5 μm (width), mucilaginous sheath absent, cell contents homogeneous, green without any granulated appearance, aerotopes absent. No heterocytes and akinetes. Cell division takes place by asymmetrical binary fission. Reproductive propagation by the help of hormogonia.

Holotype: Holotype (AP3) deposited and cryopreserved in Microbial Culture Collection (MCC), India bearing an accession number MCC3172.

Type locality: Sagar island (21.44.7N 88.7.2E) of the Indian Sundarbans, India.

Etymology: The specific epithet '*pallustris'* represents the swampy habitat of the strain from where it was collected.

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Review

Mushroom-derived polysaccharides as antitumor and anticancer agent: A concise review

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ABSTRACT

Nowadays, mushrooms with enhanced medicinal properties are being focused on finding such compounds that could modulate the immune systems of the human body. Mushrooms are extensively known for their antimicrobial, antidiabetic, antiviral, hepatoprotective, antitumor, and immunomodulatory properties owing to the presence of various bioactive components. However, a few of them are characterized and reported so far. Various polysaccharides, including β-glucans, are the principal constituent of the mushroom cell wall and play a significant role in their biological activity. This review aimed to focus on a concise report on the extraction process of the active ingredients from a mushroom with some therapeutic applications. Here, we have briefly described the medicinal properties of some commonly used mushroom extracts or their derivatives. It is interesting to note that mushroom is a potential source of many bioactive products that boost immunity. Thus, the development of functional medicinal food is essential for human welfare.

1. Introduction

Mushrooms are widely cultivated worldwide due to their excellent medicinal properties. The mushrooms can be defined as aerial umbrella-shaped macrofungi, commonly found on the forest floor. However, most of the drugs synthesized nowadays are based on chemical functionalization; some have deleterious effects on living systems. The concept of producing herbal drugs from natural sources is very primitive, authentic, and immensely important. Examples of such herbal drugs derived from natural resources may include digitoxin, morphine, progesterone, vinblastine, vincristine, taxol, etc. Unlike plant products, mushroom-derived bioactive components were also reported with enhanced bioactivity. Mushroom belongs to a large group of macrofungi, commonly known as *Basidiomycetes*, and a few from *Ascomycetes* (Moradali et al., 2007; Ferreira et al., 2010). Various bioactive compounds were extracted from mushrooms as a source of an immunomodulatory agent. *Agaricus* spp. (button mushroom), Pleurotus*spp.* (Oyster mushroom), *Lentinus* spp. (shiitake mushroom) are commonly eaten in Asian countries, such as China, Japan, and India. Most well-known species with potential medicinal properties may include *Ganoderma* (Lingzhi), Lentinus (shiitake), Auricularia, *Flammulina, Grifola* (Maitake), *Trametes and Tremella, Pleurotus, Agaricus, Clitocybe, Antrodia, Trametes, Cordyceps, Xerocomus, Calvatia, Schizophyllum, Flammulina, Suillus, Inonotus, Inocybe, Funlia, Lactarius, Albatrellus, Russula,* and *Fomes* spp (Acharya et al., 2018). Fig. 1 depicts some of the essential medicinal mushrooms that have been used as antitumor and anticancer agents.

Most of the bioactive components extracted from mushrooms are the product of secondary metabolism. These metabolites are of low molecular weight substances, principally produced in response to extracellular stress (Chaturvedi et al., 2018). It was reported earlier that mushroom-derived polysaccharides could inhibit cancer progression and therefore be recognized as an anticancer or antitumor agent. Cancer is considering the cause of second-most death worldwide after cardiovascular diseases (CVD) (Ayeka, 2018). It is estimated that death caused by cancer will be about thirteen million by 2030 (Ferlay et al., 2008; Torre et al., 2012). Cancer is a result of uncontrolled cell division and mainly spread into surrounding tissues. Such forms of cancer may result in visible growths, known as tumors, such as teratoma, leukemia, and others (Borchers et al., 2004; Zaidman et al., 2005; Ruddon, 2007).

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Fig. 1. Some of the well-known mushrooms with enhanced anticancer and antitumor properties.

Several factors, including genetic, biophysical, and biochemical processes, may induce cancer progression and metastasis. The conventional treatment strategies for cancer include the application of various chemotherapeutic drugs. However, the available procedures negatively affect patients' health; therefore, an alternative way to treat the disease is of great concern.

According to some pharmacological studies polysaccharides has found as a primary bioactive compound of mushroom (Zhang et al., 2007). Polysaccharides are bio-macromolecules forms by monosaccharide units linked together with glycosidic bonds, which may sometimes give a more complex structure (Daba and Ezeronye, 2003). The monosaccharide composition, their sugar sequence, linkage pattern, length, and nature of side-chain determine the polysaccharide structures (Tang et al., 2020a). Due to its high potential of structural variability, polysaccharides can carry the highest biological information (Wasser, 2002). The most known polysaccharides of mushrooms belong to the 1, 3-β-glucans family (Chaturvedi et al., 2018). The beta-glucan polymers often form a chain with β-(1 → 3) linkages with some occasional β-(1 → 6) linkages. Isolated naturally occurring polysaccharides of mushrooms are both neutral and acidic (Zhang et al., 2007). Some polysaccharides consist of simple chains linked by glycosidic bonds, while in more complicated forms, it binds with protein and peptide ones.

Besides the primary structure, a more complex chain structure also has important antitumor properties (Wasser, 2002). Although there is a predominantly occurrence of glucan that has been found, heteroglucan has also discovered. Glucans consist exclusively of D-glucose subunits, whereas heteroglucans consist of the side chain of monosaccharides (Zhang et al., 2007). Some most essential polysaccharides used in the field of medicine are lentinan extracted from *Lentinan edodes*, krestin from fruiting bodies of *T. Versicolor*, Schizophyllan from *S. Commune*, PSP from *Tricholoma laboyense*, polysaccharides from the fruiting body of *H. Erinaceus*, and Pleuran from *P. Ostreatus* (Zhu et al., 2015). In comparison to the total content of β-glucan highest percentage of 1, 3-β-D-glucan and 1, 6-β-D-glucan detected as follows: *G. cyanescens* (54%), *S. granulatus* (49.8%), *A. auricula-judae* (47.9%), *and S. Variegates* (40.6%). A human cannot digest the most common polysaccharides from mushrooms, such as glycan, due to its beta bond, which cannot be broken down inside the body. Nevertheless, these molecules potentially trigger the immune system to be absorbed by the gastrointestinal tract. The antitumor activity of these polysaccharides is due to their potential to stimulate immune responses via macrophage or lymphocyte. β-glucan raised the secretion of various pro-inflammatory and anti-inflammatory cytokines, NK cells, T cells, macrophages, which interact with the tumor cells. Therefore, this review briefly discusses some commonly used mushroom-derived polysaccharides and their anticancer/antitumor properties. Moreover, a detail of extraction, purification, and potential application is also well-described in this article.

2. Bioactive polysaccharides from mushrooms

Various bioactive polysaccharides isolated from mushrooms are shown in Fig. 2. Glucan is the principal constituent of mushroom polysaccharides. Two glucose monomers bind together by the α- or β-glycosidic bond in C1–C3, C1–C4, or C1–C6mannersto produce glucan chain (Pandya et al., 2018). Heteroglycans may also produce by binding arabinose, fructose, mannose, xylose, etc. Polysaccharides sometimes bind with protein or peptide and form complexes (Cui and Chisti, 2003). Most of the essential polysaccharides are products of the C1–C3 bonding of glucans. 1–6 β-D glucans. Other polysaccharides, such as lentinen isolated from *Lentinus edodes*, krestin or (polysaccharide K, PSK) derived from *T. versicolor*, polysaccharide-protein complex (PSPC) from *Tricholoma laboyense*, Pleuran from P. *ostreatus*, also polysaccharides from fruiting bodies and mycelium of *H. Erinaceus* (Zhu et al., 2015). Lentinen derived from *L. edodes* is (1 → 3)-β-glucan containing five (1 → 3)-β-glucose residues arranged in linear linkage along with two (1 → 6)-β-glucopyranoside branches inside chains gives its structure (Pandya et al., 2018). Lentine is chiefly composed of beta-glucan, which shows potential antitumor activity. The molecular weight of lentinen is about 400–800 KDa. Lentinen shows antitumor properties by enhancing cytokine secretion. Schizophyllan, another important mushroom-derived polysaccharide is having (1 → 3)-β-glucan. It contains a β-glucopyranosyl group joined by a β-(1 → 6) linkage to every third or fourth residue of the main chain. The molecular weight of Schizophyllan is about 450 kDa (Zhang et al., 2014). The polysaccharide krestin derived from *T. versicolor* (Zhu et al., 2015) has a molecular weight of 94 kDa and is a beta-glucan-protein complex. It contains acidic amino acids like aspartate, glutamate, essential amino acids like lysine arginine, and neutral amino acids like valine and leucine. For every fourth glucose unit, there are (1 → 6)-β-glucopyranosidic side chains in (1 → 4)-β-glucan (Maehara et al., 2012). Maitake D-fraction obtain from *Grifola frondosa* contains β-D-glucan with β-(1 → 6) leading chains with β-(1 → 4) branches, it also contains more β-(1 → 3) leading chains and β-(1 → 6) branches (Matsui et al., 2001a,b).

Usually, polysaccharides remain embedded with proteins forming polysaccharides protein complex, also known as PSPC. *For example, T. lobayense* consists of 54.3% polysaccharides and 35.9 % proteins. These polysaccharides are composed of galactose, glucose, arabinose, xylose, rhamnose, fucose, and mannose with corresponding proteins, such as aspartic acid, glutamic acid, serine, glycine, lysine, and threonine (Liu et al., 1995). The molecular weight is 154 *k*Da. GLPP was obtained from *Ganoderma lucidum*, which is reported as a polysaccharide-peptide. Ganderon that contains D-rhamnose, D-xylose, D-fructose, D-galactose, and D-glucose. GLPP stimulates interferon-γ (IFN-γ) and interferon-inducible protein-10 (IP-10). Ganoderon is another important polysaccharide derived from *G. lucidum*, having a molecular weight of 20 *k*Da. Similarly, G. applanatum contains several kinds of glucans with an average molecular weight of 300-1000 *k*Da (Xu et al., 2011). *G. tsugae* contains seven potential antitumor polysaccharides–protein complexes, out of which 2 are glucogalactans with similar protein content to mannose or fucose, and 5 are protein-containing (1 → 3)-β-glucan (Pandya et al., 2018).

3. Extraction and purification of polysaccharides

The extraction of polysaccharides is a crucial step for obtaining bioactive material with good quality and amount (Chen et al., 2018). There are several specialized methods for the extraction of polysaccharides (Pan et al., 2013) The selection method and purification procedure depend on the cell wall type (Mizuno, 1996). Hot water

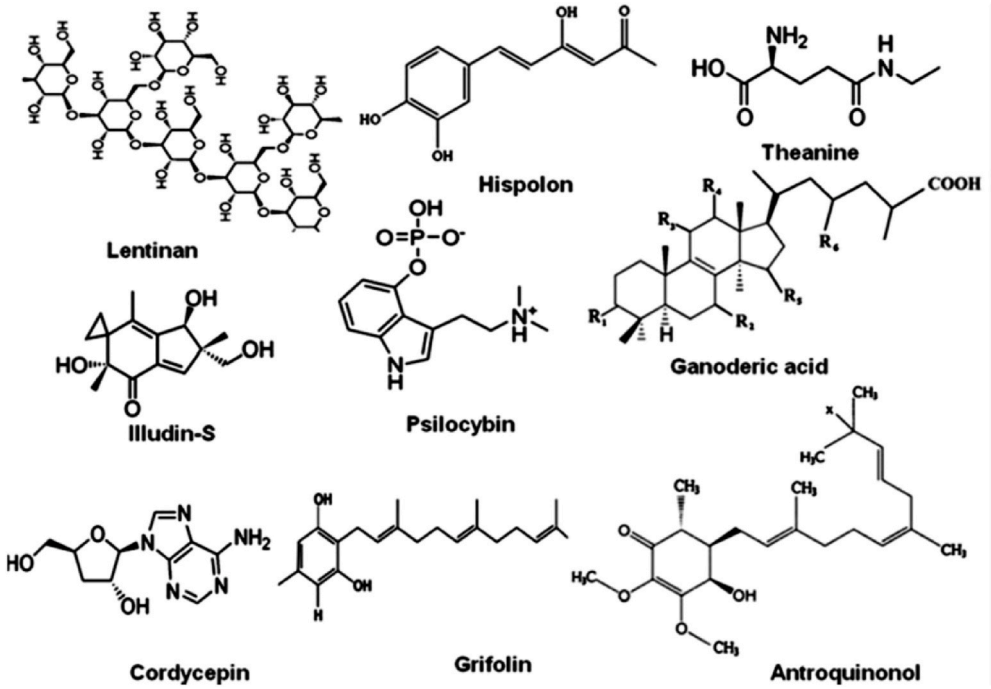

Fig. 2. Examples of some mushroom derived active components.

extraction is a very popular, easy, and standard process for extraction. According to Yan et al. (2018), fruiting bodies are mixed with 95% ethanol (w/v, 1:10) to remove fat. The residues were now extracted with distilled water (w/v, 1:20) three times at 100 for 4hrs. Extracts now precipitated using 95% ethanol (4 vol) at 4 for 12h followed by concentrated under vacuum at 60. Now centrifugation at 4000 rpm done for 15min. After collecting the precipitate, it is re-dissolved in water (Yan et al., 2018). Three successive extractions can also extract polysaccharides with water (100 for 3h),2% ammonium oxalate (100 for 6h), 5% sodium hydroxide (80 for 6h). The extraction with hot water gives water-soluble polysaccharides, and that of alkali gives water-insoluble ones. The mixture of extracted polysaccharides can be separated with a sequential extraction method (Tang et al., 2020c). Polysaccharides with different properties can be separated by cold and hot water, ammonium oxalate, HCl, Na2CO3, NaOH (2M and 4M concentration) sequentially (Colodel et al., 2018). Isolation of isolated pectin and hemicelluloses by cold water, boiling water, 1% ammonium oxalate solution, and 10% sodium hydroxide solution, successively was done by Jackson).

For retaining the acetyl group, DMSO can be used before alkali treatment during the extraction of hemicelluloses. Extraction with the only alkali may remove the acetyl group. Water at room temperature or in boiling state or followed by alkaline solutions can easily extract D-glucans (Ruthes et al., 2015). In some studies, the optimum condition for alkaline extraction was found using NaOH (0.5 mol/L) at 60°C for 2 h (Li et al., 2015 ; Jia et al., 2019). Several impurities like pigments, starch, and several small molecules may be associated with extracted polysaccharides. The impurities must be removed. Impurities like small molecules can be removed by dialysis (Du et al., 2018), ethanol precipitation (Ruthes et al., 2015; Smiderle et al., 2006), or ultrafiltration. Starch may be removed by α-amylase through the enzyme hydrolysis method (Chen et al., 2018b; Yi et al., 2019). Chloroform and n-butanol can be used to precipitate the protein molecules from polysaccharides. Although it has been reported that enzyme hydrolysis in mild doses is more effective for removing impurities (Zhang et al., 2014).

Nowadays, some modified and new techniques have arrived for the separation of proteins. Freeze-thaw techniques target the changing of buffer environment to precipitate protein (Xiong et al., 2017). Pigments can be removed from polysaccharides by various methods like anion-exchange macroporous resin (Cao et al., 2019; Wang et al., 2018a), organic solvents successive rinse and activated carbon adsorption (Chai and Zhao, 2016), hydrogen, using of peroxide (H2O2) (Chen et al., 2019; Kasipandi et al., 2019). Polysaccharides now finally fractionate and purified by various methods like gradient ethanol precipitation method (Hu and Goff., 2018), salt gradient fractionate method (Guan et al., 2015), using cetyltrimethylammonium bromide (CTAB) (Phélippé et al., 2019; Lei, 2016), ultrafiltration (Delcroix et al., 2015; Emami et al., 2018; Liu et al., 2018), ion-exchange column chromatography (Henke et al., 2019; Chen et al., 2018c), gel-column chromatography (Chen et al., 2018c; Han, 2018, Ghosh et al., 2019), affinity chromatography (Magdeldin, 2012), etc. Table 1 depicts some of the standard extraction techniques and their potential advantages and disadvantages.

4. Antitumor and anticancer properties

The β-glucans is the main compound which makes mushroom therapeutically important (Baldassanom et al, 2017; Chen et al., 2014). *Trametes robiniophila, Murill* spp., *Coriolus versicolor, Grifola frondosa, Flammulina velutipes*, and many others show potential antitumor, anticancer properties (Ayeka, 2018). Instead of directly arresting cancer cells, polysaccharides enhance the immunomodulatory effect of the host (Xu et al., 2015). In addition to chemotherapy, surgery the effects of beta-glucan can be used in immune-stimulatory and antitumor effects (Twardowski et al., 2015). *β*-glucan shows a direct inhibition effect on tumor metastasis and prevents oncogenesis, (Neergheen et al., 2020) and by inducing an immune response in the host, it shows its antitumor activity (Table 2) (Pandya et al., 2018a,b,c; Wang et al., 2017a; Hapuarachchi et al., 2017). Mushroom-derived polysaccharides and their protein complexes are significant sources of antitumor and immunomodulatory agents (Sarangi et al., 2006; Hong et al., 2004; Mizuno, 2002). The first report of the use of polysaccharides in anticancer and antitumor therapy was reported by Nauts et al., in 1946, and the use of mushroom-derived polysaccharides was reported by Chihara. The first reported mushroom-derived polysaccharide was lentinan, which was effective against both mice and human cancer cells. Fig. 3

Table 1
List of some potential antitumor and anticancer polysaccharides extracted from mushroom (Tang et al. SP 2020).

Source mushroom	Name of the polysaccharides	References
Agaricus subrufescens	glucans	Oshiman et al. (2002)
Armillaria tabescens	α-(1 → 6)-D-glucan	Luo et al. (2008)
Auricularia polytricha	(1 → 3)-linked-β-D glucopyranosyl	Song and Du (2010)
Cordyceps sinensis	Polysaccharides	Sheng et al. (2011)
Ganoderma lipsiense	exopolysaccharides, glucans	Lee et al. (2007a)
Grifola frondosa	Maitake D-Fraction	Matsui et al. (2001)
Hericium erinaceus	xylan, glucoxylan, β-glucans	Kim et al. (2011)
Lentinula edodes	Lentinan Zhang et al., 2011 β-(1 → 3; 1 → 6)-glucan Chain of (1 → 4), (1 → 3) glucanose residues with side chains of (1 → 4) glucanose	Zhang et al. (2011) Yu et al. (2010)
Lentinus polychrous	Polysaccharides	Thetsrimuang et al. (2011)
Lentinus strigellus	Polysaccharides	Lin et al. (2004)
Phellinus igniarius	Endo-polysaccharide	Yang et al. (2009) Chen et al. (2011)
Pleurotus citrinopileatus	PCP-3A (Nonlectin glycoprotein) immunomodulatory protein	Chen et al. (2010a)
Sparassis crispa	β- (1 → 3)-D-glucan	Ohno et al. (2003)
Schizophyllum commune	Schizophyllan	Hobbs (2005)
Taiwanofungus camphorates	Polysaccharides	Chen et al. (2010b)
Trametes versicolor	Polysaccharide peptide Protein bound β-(1 → 3; 1 → 6)-glucan Polysaccharide-Kureha or polysaccharide-K, krestin	Ooi and Liu (2000)
Tremella fuciformis	β-(1 → 3)-D-glucans, heteroglycans with α-(1 → 3)-mannan backbone & xylose- and glucuronic acid side chain	Bin (2010)
Tremella mesenterica	GXM (glucuronoxylomannan α-(1 → 3)-mannan)	Vinogradov et al. (2004) Wu et al., 2018
Inonotus obliquus	α-linked fucoglucomannan	Mizuno et al. (1999)

demonstrates some of the mushroom-derived anticancer polysaccharides and their applications. Udchumpisai and Bangyeekhun show that polysaccharides isolated from *Lentinus velutinus* show cytotoxic effects against cancer cells. The polysaccharides show an effective anticancer property on human HeLa and HepG2 cell lines in a time and concentration-dependent manner. The underlying mechanism of β-glucan in triggering immune response is probably through the activation of complement-component receptor-3 (CR3) that systematically induce the neutrophil or NK cells response. The CR3 is primarily expressed by myeloid cells and NK cells which induce the production of various cytotoxic granules or enzymes (Hong et al., 2004). Recently, it was reported that the β-glucan-mediated antitumor effect is mainly due to the activation of C-type lectin receptor Declin-1 (CLRD-1), activation of several APCs, and pro-inflammatory macrophages (M1 type), respectively (Alexander et al., 2018) (see Table 3).

Yukawa shows that the polysaccharides extracted from *L. edodes* have a cytotoxic effect on HepG2 cells. In this case, Polysaccharides treated cancer cells detached from the surface and became shrunken and rounded. According to Li et al. (2015), the viability of HeLa and A549 cancer cells was 0%–67.9% when treated with 600 mg/mL of polysaccharide mushrooms extracts for 48h. B. Chen (2010) shows that polysaccharides extracted from *Tremella fuciformis* show a practical inhibitory effect on HepG2 cells, and at 50 mg/mL concentration, it shows 92% antitumor property. The proliferation of $CD4^{+}$ T cells in scald mice infected by *Pseudomonas aeruginosa* can be changed by the polysaccharides extracted from *T. fuciformis*, resulting in a decline in IL-10 level (Shi et al., 2014). Chen et al. (2007) show that the skin pulp of two Bufo species showed potential antitumor property when it was treated with polysaccharides extracts from mushrooms like *Polyporus umbellatus*, *Poria cocos*. Both in vitro and in vivo experiments were performed by Weng and Yen) using *G. lucidum*, which shows anticancer property by modulation of kinase signaling. The apoptotic property of polysaccharide extract of *G. lucidum* on human gastric carcinoma cells was shown by Jang et al. (2010). Ishii et al. (2011) reported that α-(1 → 4)-Glucan-β-(1 → 6)-glucan protein complex of *A. Subrufescens* shows a potential antitumor property. Water-soluble polysaccharides extracted from king oyster mushrooms were done by Liu et al. (2015a,b). The polysaccharide shows an inhibitory effect on tumor cells and serum cytokine IL-2, TNF-α, thymus and spleen indices, and LPS- or ConA-induced lymphocytes proliferation. The potent antitumor activity in A549 cells of water extracted polysaccharides from *Fomes fomentarius* was shown by Kim et al. (2015). Polysaccharides extracted from *the Pleurotus ostreatus* mycelia component show a significant inhibitory effect on the BGC-823 human gastric cancer cell line in vitro (Cao et al., 2015). In another experiment, a water-soluble extract containing polysaccharide extracted from *P. Ostreatus* inhibits the invasion of Caco-2 cells, that is, the colon cancer cells, through the basement membrane (Cojocaru et al., 2013). Polysaccharides polysaccharide POPS-1 from *P. Ostreatus* show potential antitumor activity against HeLa cells (Tong et al., 2009). Moharib et al. (2014) show that clinically induced colon cancer in HCT-116 cells shows anti-proliferative nature after treatment with *Pleurotus Sajor-caju.* Fan et al. (2011) show that crude extract of *Agaricus brasiliensis* has an apoptosis effect on CAL-27 (human oral cancer cell). An investigation on Maitake D-fraction by Alonso et al. (2017) and Alonso et al. (2018) showed the apoptotic effect on

Table 2
Advantages and disadvantages of some common extraction procedures of polysaccharides (Tang et al., 2020a,b,c).

Purification methods	Applicable polysaccharide fractions	Advantages	Disadvantages
Gradient ethanol precipitation	Fractions with large difference on *Mw* distribution	Simple, inexpensive	Low efficiency, unhomogeneous polysaccharides after purification
Salt fractionation	Fractions with large difference on *Mw* distribution	Simple, inexpensive	Low efficiency; easy co-precipitation
CTAB method	Fractions embracing neutral and acid polysaccharides	Good selectivity for acid polysaccharide	Low efficiency, requirement of desalting
Ultrafiltration	Fractions with large difference on *Mw* distribution	Easy scale-up, high efficiency	Low yield, time-consuming
Ion-exchange column chromatography	Fractions bearing different charge strength	High purity of eluate, easy operation	Time-consuming, expensive, sometimes the height of column bed may change when buffer pH changes
Gel column chromatography	Fractions with difference on *Mw* distribution	Good separation effect, mild condition	Expensive, inefficient, hard for scale-up
Affinity column chromatography	Fractions having matched ligand	High purity, few steps	Difficult to find a proper ligand for a given polysaccharide

Table 3
An overview of some commonly used mushrooms as immunomodulatory agents with detailed emphasis.

Name of the mushroom	Bioactive compound	Source	Antitumor and anticancer activity	Reference
Agaricus bisporus	α-glucans and β-glucans	fruiting body	Has potent Immunomodulation properties	Kozarski et al. (2011)
Boletus edulis	Heteropolysaccharide with a Heterogeneous main chain	fruiting body	Potent immunomodulatory effect	Wang et al. (2014)
Dictyophora indusiata	Heteroglycan, mannan, glucan from.	fruiting body	Antitumor activity	Liao et al. (2015) Thekkuttuparambill et al., 2007
Pleurotus ostreatus	polysaccharides 2 (POMP2), polysaccharides -1(POPS-1).	mycelium	Anticancer activity	
Cordyceps militaris	β-glucan.		Inhibition of IL-1β, TNF-α, and COX-2 expression	Smiderle et al. (2014)
Pleurotus tuber-regium	β-D-glucan	Sclerotium, mycelium	anti-breast cancer activity	Zhang et al. (2006)
Inonotus obliquus	Glucan	Fruiting body, mycelium	Antitumor, immunomodulation	Kim et al. (2005)
Pleurotus citrinopileatus	Galactomannan	Fruiting body	Antitumor	Wang et al. (2005)
Phellinus linteus	Glucan	Fruiting body	Antitumor	Kim et al. (2004)
Polypours umbellatus	Glucan	Mycelium	Antitumor, immunomodulation	Yang et al. (2005)
Agaricus blazei	Fruiting body; Mycelium	Glucan, heteroglycan, glucan protein, glucomannan-protein complex	Antitumor activity	Mizuno (1995)
Agaricus bisporus	Fruiting body	α-glucans and β-glucans.	Immunomodulation and antioxidative activities	Kozarski et al. (2011)
Auricularia auricula-judae	(1 → 4)-linked D-glucopyranosyl main chain with (1 → 6)-linked D-glucopyranosyl branch at O-6	Fruiting body.	Antitumor activity	Xu et al. (2012)
Boletus edulis	Heteropolysaccharide with a heterogeneous main chain (Glc*p*, Gal*p* and Rha*p*)	Fruiting body.	Immunomodulatory activity	Wang et al. (2014)
Calocybe indica	Heteropolysaccharide with a heterogeneous main chain (Gal*p* and Glc*p*)	Fruiting body	Immunomodulatory andcytotoxic activities	Mandal et al. (2011)
Dictyophora indusiata	Heteroglycan, mannan, glucan	Fruiting body	Antitumor and hyperlipidemia activity	Liao et al. (2015)
Ganoderma applanatum	Glucan	Fruiting body	Antitumor activity	Nakashima et al. (2013)
Ganoderma lucidum	hetero-β-D-glycans (glucurono-β-D-glucan, arabinoxylo-β-D-glucan, xylo-β-D-glucan, manno-β-D-glucan and xylomanno-β-D-glucan)	Fruiting body	Induced cell-cycle arrest and apoptosis	Zhang et al. (2010)
G. frondosa	β-glucan		stimulates differentiation of haematopoietic progenitor cells, production of granulocyte colony-stimulating factor, and the recovery of peripheral blood leukocytes	Wesa et al. (2015)
Coriolus versicolor	glucan	fruiting bodies	novel antitumor	Awadasseid et al. (2017)
Pleurotus pulmonarius	Beta-glucan		Inhibit the leukocyte migration to injured tissues. Mice previously treated with beta-glucan showed a reduction of writhes	
Trametes versicolor	glucan		improved survival and immune function in human randomized, controlled trials in cancer patients	
Coriolus versicolor	polysaccharide K (PSK)		increased the survival of patients after curative gastric cancer resection over chemotherapy alone	Oba et al. (2007)
Amauroderma rude	polysaccharide		inhibited tumour growth in mice via regulation of the immune system at the molecular and cellular levels	Chang et al. (2015)
Hericium erinaceus	Polysaccharide		prevented migration of cancer cells of implanted colon tumors in mice to the lung	Li et al. (2015)
Coriolus versicolor	polysaccharides		Shows anticancer property and shows anti-metastasis effects on mouse mammary 4T1 carcinoma	Zhu et al. (2014)
Phellinus linteus	Hispolon Water-soluble polysaccharide (POPS-1)		Induced apoptosis of breast- and bladder-cancer cell) Exhibited significantly lower cytotoxicity to human embryo kidney 293T cells than HeLa tumour cells compared with anticancer drug 5-fluorouracil	Lu et al. (2009)
Cordyceps taii	Chlrophorm extract		has potent in vivo antitumor and antimetastatic activities	Liu et al. (2015)
Ganoderma formosanum	PS-F2, a polysaccharide fraction		Worked as stimulant for tumour-specific cellular and humoral immune responses	Wang et al. (2014), Baldassanom et al. (2017)

(*continued on next page*)

Table 3 (*continued*)

Name of the mushroom	Bioactive compound	Source	Antitumor and anticancer activity	Reference
Ganoderma lucidum	GP-1 and GP-2 types of polysaccharides		Increased the proliferation and pinocytic activity of macrophage significantly and inhibited effect on the cancer cell	Zhao et al. (2010)
Sarcodon aspratus	Two polysaccharide fractions (PSAN and PSAA)		At a concentration of 400 mg/L and an exposure time of 24 h, the inhibition rates for PSAN and PSAA were 65 and 80%, respectively	Chen et al. (2013)

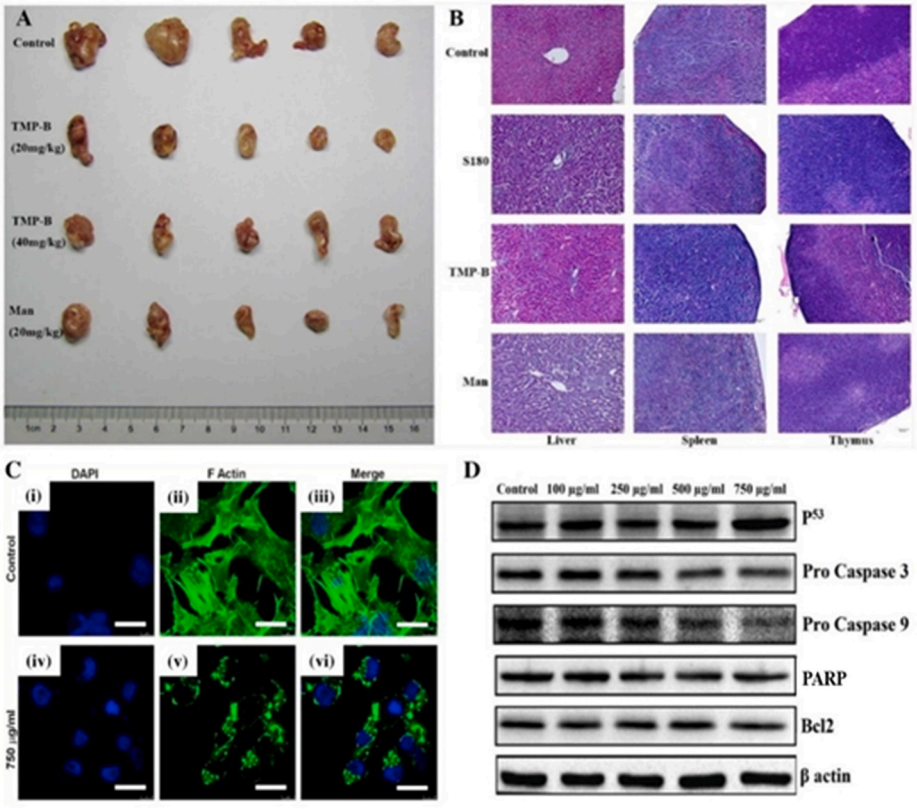

Fig. 3. *In vitro* and in vivo antitumor activity of mushroom-derived polysaccharides. **(A)** Effect of *Tricholoma matsutake*-derived polysaccharides (TMP-B) on relative tumour volume of Kunming male mice after 4 weeks' post-administration. **(B)** Haematoxylin & Eosin (H&E) staining indicating the tissue sections of liver, spleen, and thymus after administration of TMP-B as compared to the control group (Sanyal and Ghosh, 2019). **(C)**Confocal laser scanning microscopy (CLSM) images of *HeLa* cells after treatment with *Lenzites betulina* extracts at indicated concentrations resulting in the change of cell morphology (Sanyal and Ghosh, 2019). **(D)** Western blotting of major apoptosis related proteins expressed in *L. betulina* treated *HeLa* cells (Sanyal and Ghosh 2012).

MDA-MB-231 cells, which in turn effective against breast cancer. Da Silva et al., 2012 showed that the isolated polysaccharide from *Macrocybe titans* showed the anticancer property in vitro in murine melanoma cells B16–F10. Migration of cancer cells stopped by the effect of polysaccharides. Polysaccharides extracted from *Collybia radicata* also show potential immunomodulatory effects (Wang et al., 2018). Under both in vitro and in vivo conditions, the polysaccharide extracted from Pleurotus pulmonarius suppresses PI3K/AKT signaling pathway actives Myr-AKT, which shows anti-proliferations in hepatocellular carcinoma (S. Xu et al., 2012). polysaccharide from *Grifola frondosa* when taken orally, the immune system of breast cancer patients stimulated (Fortes et al., 2008). Beta-glucan lentinan increases the survivability of patients with prolonged gastric cancer (Deng et al., 2009). Polysaccharides (GTM1 to GTM6) isolated from *Ganoderma* by Peng et al. exhibited superior antitumor property by increasing activity of NK cells and cytotoxic T-lymphocytes. Polysaccharide extract from mycelium biomass of *Pleurotus ostreatus* shows inhibitory effect on Ehrlich Tumor (ET) and Sarcoma 180 (S180) cells (Pauliuc et al., 2013). Cao et al. demonstrated that *Ganoderma lucidum* polysaccharides-peptide (GLPP) shows an anti-angiogenic effect. The GLPP stimulates interferon-γ, interferon-inducible protein 10, and interleukin-12 level.

5. Mode of action

Previous studies indicate that β-glucans themselves have no significant cytotoxicity in the human body. However, it stimulates monocyte recruitment and acts as an immunomodulant. In addition, β-glucan and ganoderic acid extracted from *G. lucidum* showed direct anticancer effects via activating the host-specific tumor immune responses through activation of pro-inflammatory macrophages (M1 type) and subsequently killing of HepG2 cells. Many mushroom-derived active compounds act through binding with pattern recognition receptors (PRRs) to stimulate immune responses, such as α-glucan, β-glucan, β-fructan, mannan, and chitosan (Jin et al., 2018). Fig. 4 briefly illustrates an overview of the possible mechanism of host-induced tumor immune responses. An ideal immunomodulator with enhanced anticancer property mainly stimulated by complement receptor-3 (CR-3) or macrophage-1, which trigger dectin-1-My-D88 mediated cytokine secretion and targeted killing of cancer cells. Mushroom-derived β-glucan also acts through the CR3 mediated My-D88 pathway. Therefore, mushrooms act as a natural antibody against certain malignant tumors by initiating tumor-specific host immune responses (Jin et al., 2018). Table 4 represents some of the examples of β-glucan that act as adjuvants in anticancer therapy.

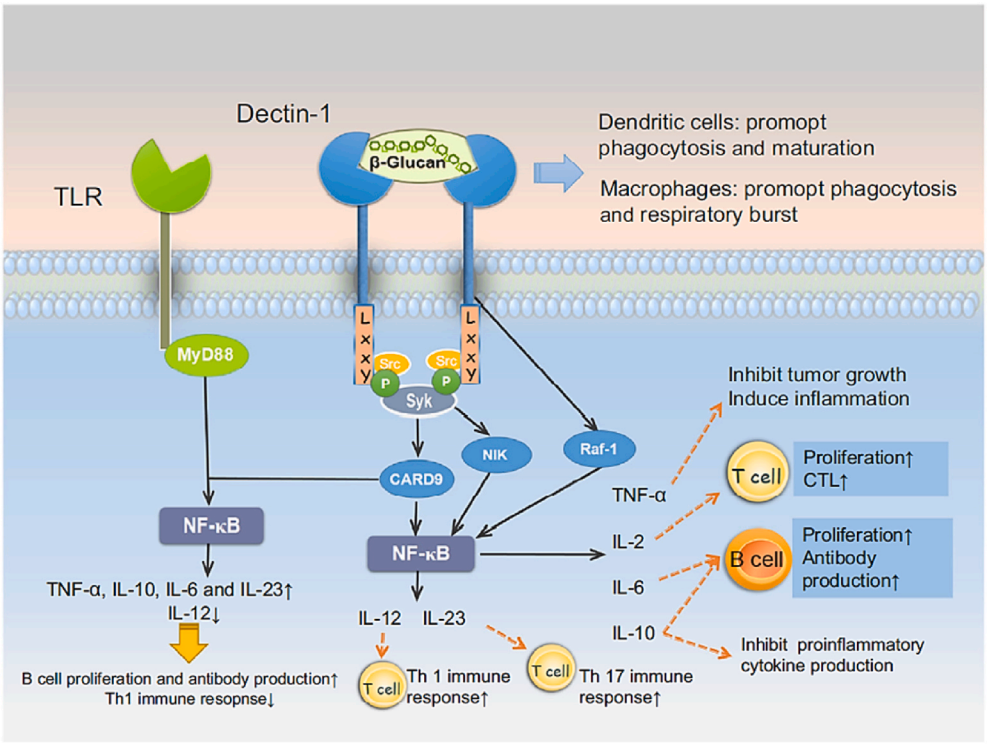

Fig. 4. Signal pathway of β-glucan binding to dectin-1 initiates the activation of various factors during immunomodulation. After binding of β-glucan with declin-1, it activates spleen associated tyrosine kinase (*Syk*) which trigger the nuclear factor kappa-beta (NF-*k*B) transcription factor through caspase recruitment domain family proteins (CARD9) or mitogen-activated protein kinases (NIK) to produce IL-10, IL-2, IL-23, IL-6, and TNF. The activation of these cytokines induce the proliferation of T and B cells along with DCs. Interestingly, when declin-1 and MyD88 are activated, a series of other signaling pathways were triggered. As a result of that various cytotoxic factors secreted form the T, B, and DCs, which initiate the host-specific tumor immune responses (Jin et al., 2018).

Table 4
An overview of β-glucan action as adjuvants in anti-cancer therapy (Jin et al., 2018).

Subjects	β-glucan origin	Administration route	mAb/vaccine	Administration route of mAb or vaccine	Tumor cells	Inoculation route of tumor cells	Effects
BALB/c and C57BI/6 mice	Yeast	Orally	*Anti*-G_{D2} mAb or anti-MUC1 mAb	Intravenously	RMA-S-MUC1 cells	Mammary fat pad (subcutaneously)	Tumor regression in all models, tumor-free survival occurred in models of stable expression of target antigen
C57BI/6 mice	Yeast	Intravenously	*Anti*-$_{GD2}$ mAb	Intravenously	RMA-S-MUC1 cells	Mammary fat pad (subcutaneously)	Tumor regression and survival
Several combined immunodeficiency mice	Yeast	Intravenously	Bevacizumab	Intravenously	SKOV-3 cells	Mammary fat pad (subcutaneously)	The therapeutic efficacy mediated by bevacizumab
Human	Yeast	Orally	Bivalent gangliosides vaccine	Subcutaneously	Neuroblastoma	N/A	Antibody responses against GD2 and/or GD3 in 12 of 15 patients; Assessment of minimal residual disease are disappeared in 6 of 15 patients
BALB/c mice	Yeast	Orally	Survivin peptide vaccine	Subcutaneously	A20 cells	Left flank (intradermally)	The number of macrophages, DC and IFN-c-secreting $CD^{8+}T$ cells increased; tumor size decreased
BALB/c mice	Maitake mushroom	Intraperitoneally	DC vaccine	Intraperitoneally (stimulated with β-glucan)	Colon-26 sarcoma cells	N/A	Expression of maturation markers on DC gradually increased; DC ability to induce antigen-specific T cell responses; Antitumor efficacy of DC vaccines enhanced
BALB/c mice	Yeast	Orally	N/A	N/A	Ehrlich ascites tumor cells	N/A	Resistance to tumour-induced immune suppression via enhancing secretion of colony-stimulating factors, IL-1α, IL-6 and IFN-γ
BALB/c mice	Yeast	Orally	N/A	N/A	Ptas-64 cells	Mammary fat pad (subcutaneously)	Conversion of non-protective Th2 response to protective Th1
C57BI/6 mice	Yeast	Orally	N/A	N/A	RMA-S-MUC1 cells	N/A	Tumor size decreased

6. Conclusion

Mushrooms have been considered a significant amount of interest from the scientific community due to their high nutritional and medicinal values. It is frequently used as a food supplement that induced the immunity of individuals through their different bio-active components. Notably, the decrease of *M. tuberculosis* bacteria in the presence of mushrooms extract enables a new possibility to treat TB disease in place of commercially available medicines that have some cytotoxic issues. In addition, a significant decrease in cell viability of tumor cells has occurred in the presence of mushroom-derived polysaccharides indicated their antitumor activity. Therefore, mushroom extracts have the potential to use as biomaterials for therapeutic applications.

Declaration of competing interest

The authors declare no competing financial interests.

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'এবং মহুয়া'-বিশ্ববিদ্যালয় মঞ্জুরী আয়োগ(UGC-CARE list-I 2021)
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এবং মহুয়া

(বাংলা ভাষা, সাহিত্য ও গবেষণাধর্মী মাসিক পত্রিকা)

২৩তম বর্ষ, ১৩৮ সংখ্যা
সেপ্টেম্বর,২০২১

সম্পাদক
ড. মদনমোহন বেরা

সহসম্পাদক
পায়েল দাস বেরা
মৌমিতা দত্ত বেরা

যোগাযোগ :
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মো.-৯১৫৩১৭৭৬৫৩

কে.কে. প্রকাশন
গোলকুঁয়াচক, মেদিনীপুর, পশ্চিমবঙ্গ।

নারীর কলমে বাংলা উপন্যাসের বিবর্তন : সূচনা থেকে প্রাক্-স্বাধীনতা

ড. শান্তনু ভট্টাচার্য

'আমি নারী, আমি মহীয়সী
আমারে স্মরি সুর বেঁধেছে
জ্যোৎস্না-তারায় নিদ্রাবিহীন শশী।
আমি নইলে মিথ্যা হত সূর্য চন্দ্র ওঠা,
মিথ্যা হত কাননে ফুল ফোটা।'

— রবীন্দ্রনাথ ঠাকুর

নারী এবং পুরুষের যৌথ জীবন যাত্রায় নারীর কাছে শিক্ষার আলো অনেক পরে পৌঁছেছে। পুরুষ প্রথম থেকেই যে সুযোগ সুবিধা পেয়ে এসেছে নারী তা পায়নি। শিক্ষার আলোয় আলোকিত হচ্ছে যখন পুরুষ, নারী তখন পারিবারিক গার্হস্থ্য কর্তব্য পালনেই সীমাবদ্ধ থাকছে। পরবর্তীকালে সময়ের সঙ্গে সঙ্গেই পরিস্থিতিও বদলেছে। উনিশ শতকের নবজাগরণের আলোকে উদ্ভাসিত হয়েছে নারী এবং পুরুষ উভয়েই। তবে পুরুষের ক্ষেত্রে সবকিছু যতটা সহজ ছিল, নারীদের ক্ষেত্রে তা ছিল না। সাহিত্য ক্ষেত্রেও প্রথমদিকে অনুরূপভাবেই পুরুষের তুলনায় নারীর পদচারণা ছিল অনেক কম। প্রাক্-আধুনিক বাংলা সাহিত্যে হাতে গোনা কয়েকজন নারী সাহিত্যিককে পাওয়া যায়। আধুনিক যুগের সূচনা পর্বেও নারী সাহিত্যিকের সংখ্যা পুরুষের তুলনায় অপেক্ষাকৃত কম। বর্তমানে অবশ্য সেই ব্যবধান অনেক কমেছে এবং প্রায় নেই বললেই চলে।

আমার আলোচ্য বিষয় নারীর কলমে লেখা বাংলা উপন্যাস, প্রথম থেকে প্রাক্-স্বাধীনতা কালপর্ব পর্যন্ত। এই সময় পর্বে বেশকিছু মহিলা ঔপন্যাসিক বাংলা উপন্যাস লিখছেন। যাদের লেখায় মূলত দুটি দিক ধরা পড়ছে। পারিবারিক গার্হস্থ্য জীবনচিত্র, যা মূলত পারিবারিক দাম্পত্য সমস্যা এবং তার সমাধান কেন্দ্রিক এবং আধুনিক শিক্ষায় শিক্ষিত মধ্যবিত্ত নারী সমাজের জীবন সংগ্রামের কথা-যাদের পারিবারিক জীবন এবং বাইরের জীবনের সঙ্গে সামঞ্জস্য রক্ষা করতে হয় প্রতিনিয়ত। পরিবারের মধ্যেই যেহেতু বেশিরভাগ সময় নারীরা আবদ্ধ থাকেন তাই পারিবারিক জীবনের খুঁটিনাটি সূক্ষ্মাতিসূক্ষ্ম বিষয়ের বর্ণনা, চাওয়া-না-পাওয়া কিংবা বঞ্চনার বিষয়গুলি বারবার ঘুরে ফিরে আসে মহিলা ঔপন্যাসিকদের লেখনিতে। উনিশ শতকের শিক্ষার আলোয় আলোকিত হয়ে নারীমন সমকক্ষতা চায় পুরুষের। পায়ের নীচে নয় পাশে অবস্থান চায়। সমস্ত কাজে

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রবীন্দ্র-দৃষ্টিতে রবীন্দ্র-কাব্য

ড. অরুণাভ মুখার্জ্জী

সারসংক্ষেপ :

কবিগুরু রবীন্দ্রনাথ তাঁর প্রতিভার ঐশ্বর্যে প্রত্যক্ষ বা পরোক্ষভাবে সর্বচিত্তে বিরাজিত– 'সবার হৃদয়ে রবীন্দ্রনাথ'! তাই নানান জনের দৃষ্টিতে অনন্য রবীন্দ্রনাথ নানানভাবে বিশ্লেষিত হয়েছেন এবং হচ্ছেনও। কিন্তু কবি নিজেকে, নিজের সত্তাকে, নিজের কাব্যকে অর্থাৎ নিজের সৃষ্টিচক্রকে কীরূপে দেখেছেন, কালাশ্রয়ী অন্তর্জীবনের বিবর্তনের ক্রমিক রূপান্তর ও বিকাশের মধ্যে দৃশ্যমান জগতে রূপ এবং সীমাতে আবদ্ধ না থেকে তাকে ছাড়িয়ে যাওয়ার এক মহৎ আবেদন রবীন্দ্রদর্শনে নিজেকে কীভাবে ব্যক্ত হয়েছে, তাঁর কাব্যসমগ্র বিশ্লেষণে সে বিষয়েই পক্ষাবলোকন করার প্রয়াস নেওয়া হয়েছে আলোচ্য রচনায়।

সূচক শব্দ :

আপনার অভিজ্ঞান, চক্রপথ প্রদক্ষিণ, আমি কবি মাত্র, সৌন্দর্যানুভূতি, ঐকান্তিক অন্তর্মুখীন, বস্তুনির্মাণক্ষমপ্রজ্ঞা, সমগ্রতা, গঙ্গাজলে গঙ্গাপূজা, কপিবুকের কবিতা, হৃদয় অরণ্য, স্বকীয় ভাব, জীবনদেবতা, আস্তিক্যবাদী।

প্রতিপাদ্য বিষয় :

রবীন্দ্রজীবন চক্রের বিচিত্র অভিজ্ঞতা নানা কর্মের উপলক্ষে নানা জনের নিকট নানাভাবে আলোচিত হয়েছে। কিন্তু তাতে যে রবীন্দ্র পরিচয়ের সমগ্রতা নেই, তা রবীন্দ্রনাথ নিজেই উপলব্ধি করতে পেরেছিলেন। আর তাই তিনি নিজেকে ব্যক্ত করতে নিজের সম্পর্কে জানিয়েছেন– "নিজের সত্য পরিচয় পাওয়া সহজ নয়। জীবনের বিচিত্র অভিজ্ঞতার মূল ঐক্যসূত্রটি ধরা পড়তে চায় না। ... নানাখানা করে নিজেকে দেখেছি, নানা কাজে প্রবর্তিত করেছি, ক্ষণে ক্ষণে তাতে আপনার অভিজ্ঞান আপনার কাছে বিক্ষিপ্ত হয়েছে। জীবনের সেই দীর্ঘ চক্রপথ প্রদক্ষিণ করতে করতে বিদায়কালে আজ সেই চক্রকে সমগ্ররূপে যখন দেখতে পেলাম তখন একটা কথা বুঝতে পেরেছি যে, একটি মাত্র পরিচয় আমার আছে, সে আর কিছুই নয়, আমি কবি মাত্র"[১]। সুতরাং রবীন্দ্র অনুভবে রবীন্দ্রনাথ ঠাকুরের প্রথম এবং প্রধান পরিচয় তিনি কবি - সৌন্দর্যানুভূতির কবি। ধর্মও ছিল তাঁর কবির ধর্ম। জীবনের বারো বছর বয়স থেকে শুরু করে মৃত্যুর পূর্ব সময় অর্থাৎ একাশি

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ফোন - ৯৮০৪৯২৩১৮২
সার্বিক সহয়তা - সৌরভ বর্মন
ফোন - ৮২৫০৫৯৫৬৪৭

মুদ্রণ
অনন্যা
বুড়ো বটতলা, সোনারপুর, কলকাতা - ৭০০ ১৫০
ফোন - ৯১৬৩৯৩১৪৬৫

মূল্য : ৬৫০ টাকা

নজরুল কাব্যে পুরাণ ভাবনা

অরুনাভ মুখার্জ্জী
সহকারী অধ্যাপক, বাংলা বিভাগ
অচ্ছুরাম মেমোরিয়াল কলেজ, ঝালদা, পুরুলিয়া

সারসংক্ষেপ : *নিখিল বাংলার চারণকবি' কাজী নজরুল ইসলামের চিন্তা-চেতনাতে পুরাণ ভাবনার স্বরলিপি সুর হয়ে প্রতিধ্বনিত হয়েছে বারে বারে। বিংশ শতাব্দীর এক অসম্ভবের সম্ভাবনার যুগে পরাধীনতার অন্তর্জ্বালা বুকে নিয়ে 'একহাতে বাঁকা বাঁশের বাঁশরী, আর হাতে রণতুর্য' এর মধ্যদিয়ে নজরুলের আবির্ভাব। বিদ্রোহ, প্রেম, মানবতা, উল্লাস, আবেগ-উন্মাদনা প্রকাশের অকুণ্ঠ প্রয়াসে তিনি এত সাবলীল ভাবে পৌরাণিক মিথকে ব্যবহার করেছেন, যা বহুল পরিমাণে আমাদের কাছে 'অ-চর্চিত'ই থেকে গেছে। আর বাংলা সাহিত্যের ইতিহাসে এক বিরল প্রতিভার অধিকারী কাজী নজরুল ইসলাম তাঁর জীবনে ও সাহিত্যে পুরাণের প্রত্যক্ষ ও পরোক্ষ প্রভাবকে কীরূপ আত্মীকরণ করেছিলেন, সেই আপাত 'অ-চর্চিত' বিষয়কে 'চর্চার' আলোকে উদ্ভাষিত করতেই আলোচ্য রচনায় অবতারণা।*

মূলশব্দ : *সামূহিক চৈতন্য, উপনিবেশ-শৃঙ্খলিত, বৈশ্বিক প্রতিক্রিয়া, শিবতত্ত্ব, ভূমিলীন মানবমহিমা, জীবনাদর্শ, সামন্ততান্ত্রিক সমাজ, সমবায়ী ঐতিহ্য, জীবনবেদ।*

মূল বিষয় :

বর্তমান শতাব্দীর ছিন্নমূল, অস্তিত্বচেতন, দ্বন্দ্বজর্জর এবং সংগ্রাম-মুখর সময় প্রেক্ষিতে পুরাণ বা মিথ ভাবনা একটি গুরুত্বপূর্ণ প্রাসঙ্গিক বিষয় সন্দেহ নেই। কারণ ব্যক্তি প্রতিভা অন্তর্গূঢ় সত্তায় ও অন্ধকার আবর্তনে শুধু নিজস্ব অভিজ্ঞতা ও অভিজ্ঞান-ই বহন করে না, সৃষ্টি মুহূর্তে তাঁর হৃদয় হয়ে ওঠে সামূহিক চৈতন্যের আধার। পুরাণ এই সামূহিক চৈতন্যেরই অংশ – "একটি চেতনা, যা মানুষের কল্পনা এবং অভিব্যক্তি প্রকাশের সঙ্গে জড়িত। এতে অতীতের স্মৃতি, বর্তমানের অভিজ্ঞতা ও ভবিষ্যতের আদর্শ সব মিলিয়ে এমন একটি প্যাটার্ন সৃষ্টি হয়েছে, কল্পনা যেখানে বস্তুভিত্তিক এবং প্রত্যয়গ্রাহ্য হতে পারে"[১]।

সাহিত্য রচনায় মিথ চেতনা তথা পৌরাণিক ভাবনার প্রয়োগ একটি বিদগ্ধ রীতি বলেই সর্বদেশে ও সর্বকালে স্বীকৃত। সমকালীন জীবন ও বাস্তব পরিপ্রেক্ষিতের মধ্যে সাহিত্য-প্রতিভা যখন তার অন্তর্গূঢ় অভীপ্সার রূপায়ণ অসম্ভব বলে মনে করেন, তখন

शैलेश मटियानी

जीवन और साहित्य के विविध आयाम

अरविन्द कुमार 'मौर्य' डॉ0 नम्रता जैन

जे.टी.एस. पब्लिकेशन्स, दिल्ली

शैलेश मटियानी जीवन और साहित्य के विविध आयाम

सम्पादक
अरविन्द कुमार 'मौर्य', डॉ० नम्रता जैन

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शैलेश मटियानी की कहानियों में महानगरीय हाशिये का समाज

गौतम सिंह राणा
सहायक प्राध्यापक
हिंदी विभाग
अछुराम मेमोरियल कॉलेज, झालदा
जिला – पुरुलिया(प. बं.)

हिंदी कहानीकारों में शैलेश मटियानी एक सुपरिचीत नाम है । हिंदी कथा साहित्य में प्रेमचंद की परंपरा को आगे बढ़ानेवाले मुकम्मल कथाकारों में निर्विवाद रुप से इनका नाम सबसे आगे लिया जाता है क्योंकि प्रेमचंद के बाद हिंदी कहानी-भंडार को अगर किसी ने अपनी विपुल सृजनात्मक वैभव से सम्पन्न किया तो वे शैलेश मटियानी ही हैं । इनके रचना-भंडार में मौजूद ३० कहानी-संग्रह इसका प्रमाण है । ऐसा भी नहीं है कि प्रेमचंद के बाद केवल कहानियों की संख्या के आधार पर इनको यह पद दिया गया है, बल्कि कहानियों की विपुल संख्यकता के बावजूद भी इनमें कहीं भी एकरसता एवं विषय की पुनरावृत्ति का दोष नहीं मिलता है । इसके अतिरिक्त इन्हें प्रेमचंद के बाद उनकी परंपरा का सबसे बड़ा कथाकार मानने का एक तर्क यह भी है कि इन्होंने अपने समय में कथा लेखन में नवीनता के लिए अन्य समकालीन अधिकांश कथाकारों की तरह अनुभवों और विचारों की जूठन बटोरने के लिए विदेशों में भ्रमण नहीं किया बल्कि प्रेमचंदीय परंपरा के अनुरूप एक भारतीय कथा-परंपरा की खोज में जुटे रहने का काम किया ताकि उन कहानियों के साथ-साथ भारतीय जीवन के निरंतर बदलते हुए य़थार्थ और जातीय स्मृति का खजाना भी सुरक्षित होता चला जाए – " यही समझ में आता है कि जिस वक्त हिंदी के ज्यादा कथाकार अनुभवों और विचारों की जूठन बटोरने के लिए विदेशों की ओर भाग रहे थे, उस समय मटियानी यहाँ की मिट्टी के दुखों और गौरव को छानते हुए, उनके बीच किस तरह एक भारतीय कथा-परंपरा की खोज में जुटे थे और उन्होंने जो कुछ लिखा, उसमें कहानियों के साथ-साथ भारतीय जीवन के निरंतर बदलते हुए यथार्थ और जातीय स्मृति का कितना बड़ा खजाना सुरक्षित होता गया है । " 1

कथाकार अपने समाज का कुशल चितेरा होता है | वह अपने इर्द-गिर्द के समाज को सम्पूर्ण संवेदना के साथ अपनी रचनाओं में चित्रित करता है | वह एक ओर समाज से संवेदित होता है तो दूसरी ओर अपनी रचनाओं से समाज को विरचित व संवेदित करने का भी महत्वपूर्ण कार्य करता है | तिसपर जब बात उस कथाकार की हो, जिनमें बाबा नागार्जुन को मैक्सिम गोर्की दिखता हो तो उनकी कहानियों में संवेदना की सांद्रता का आधिक्य स्वतः ही सिद्ध हो जाता है । किसी कथाकार की कहानी में संवेदना की सांद्रता का आधिक्य का उद्रेक ऐसे ही आनायास नहीं हो जाता है बल्कि दम तोड़ती जीवन से संघर्ष कर किसी तरह जी लेनेवाले समुदाय के साथ रहकर मिले जीवनानुभूति से ही संभव हो पाता है और इस जीवनानुभूति से शैलेश मटियानी संपृक्त थे । शैलेश मटियानी की कहानियों का अनुशीलन करने पर हम पाते हैं कि उनकी कहानियों में मूलत: दो समाज रचता-बसता है- 1. कुमायूँ का पार्वत्य-समाज 2. बम्बई-महानगरीय हाशिये का समाज । इस संबंध में उनका खुद का ही कहना है - "एक ओर मेरा अभिष्ट कुमायूँ के जनजीवन, वहाँ की संस्कृति और लोक-साहित्य को उनके अस्तित्व और उनकी आत्मा के अनुरूप शब्द-शिल्प देकर, उन्हें हिन्दी साहित्य के विशाल सागर तक ले आना... तो दूसरी ओर मैं बम्बई के फुटपाथों, कमाठीपुरा के कोठों में चिलबिलाने-बिलखनेवाली बेटियों और गणपत रमन्ना भाऊ तथा उस्ताद पोपटों की अपनी बिरादरी के प्रति वैयक्तिक और साहित्यिक दायित्वों के साथ बँधा हुआ हूँ। " 2

कुमायूँ के पार्वत्य समाज में जन्मे व बाल्यकाल तक पले-बढ़े शैलेश मटियानी को लेखक बनने की चाह के बरक्स अपना गुजारा करने के लिए काम की तलाश ने मुंबई महानगर के गजालत से भरे हाशिये के समाज में लाकर खड़ा कर दिया था । इसके बावजूद भी उनके लेखक बनने की चाह कभी भी खत्म नहीं हुई क्योंकि उनका मानना था – " कोई लेखक जितने कष्ट सहता है, उतना ही बड़ा उसका लेखन होता है । "3 कहने का तात्पर्य है कि लेखक का वास्तविक जीवनानुभव जितना विशाल होगा, उसका लेखन उतना ही उच्च कोटि का एवं विविधतापूर्ण होगा । यही कारण है कि पार्वत्य समाज के साथ-साथ मटियानी ने मुंबई महानगर में अपने द्वारा भोगे हुए यथार्थ व बहुत करीब से देखे हुए सर्वहारा वर्ग के समाज को भी अपनी कहानियों में काफी जीवंतता के साथ चित्रित भी किया है | सर्वहारा वर्ग कहने का तात्पर्य समाज के हाशिये पर जी रहे चोर-उचक्के, पाकेटमारों, भीखमंगों के समाज से हैं, जिसके बीच मुंबई में उनका जीवन कटा था । इस वर्ग के समाज को चित्रित करनेवाली मटियानी की प्रमुख कहानियों में 'मिट्टी', 'प्यास',

'भय', 'इब्बूमलंग', 'दो दुखों का एक सुख', 'एक कोप चा : दो खारी बिस्किट', 'चील', 'महाभोज', 'रहमतुल्ला','अहिंसा', 'इल्लेस्वामी' आदि हैं।

'मिट्टी' कहानी एक भिखमंगे(टुंडे लालमन) और उसके साथ रहनेवाली गनेसी की है। लालमन बिमार है। गनेसी उसे हाथठेले में बिठाकर भीख माँगती है। अपनी बीमारी के कारण लालमन लगातार छीज रहा है। उसे बार-बार निबटने की जरूरत महसूस होती है ; परन्तु इस हालत में भी उसकी स्वादिष्ट भोजन खाने की इच्छा कम नहीं होती। गनेसी परेशान है। जितना कुछ भीख में मिलता है उससे लालमन की दवाई एवं खाने-पीने का खर्च किसी तरह निकल जाता है। इस कारण गनेसी कल्पित चिनी की बिमारी का खाका लालमन के आगे खिंचती है ताकि उसका दही-जलेबी खाना छूट जाए। यह प्रेम की अतिशय करुणा और स्थितियों के त्रास से उपजी विरक्ति की कहानी है। इसमें एक ओर तो हिन्दुस्तान के गजालत से भरे तबके का यथार्थ है जो लंबे समय से आजाद भारत की सच्चाई की कलई खोलकर हमारे सामने रख देता है तो दूसरी ओर गनेसी जैसे गरीब पात्रों की चारित्रिक उज्जवलता को भी प्रेषित करता है।

'प्यास' कहानी एक जेबकतरे शंकरिया की कहानी है जिसे जिंदगी के आभाव और अंतहीन तकलीफों ने अपराध की दुनिया में धकेल दिया है। अब वह एक जेबकतरा बन गया है। इस पर भी उसकी तकलीफ खत्म नहीं होती है। एक तरफ तो उसे जेबकतरा नाम की गाली को ढोने का कष्ट है तो दूसरी ओर जेबकतरई के दौरान मिली पिटाई की तकलीफ भी। एक बार सोने की चैन झपटने के दौरान पकड़े जाने और बेदम पब्लिक पिटाई के बाद जब वह अपने को बचाने के उद्धेश्य से स्वयं को पलिस के हवाले कर देता है तो फिर उसे पुलिस-तंत्र का दिल दहला देनेवाला घोर अमानवीय व्यवहार को झेलना पड़ जाता है। वह अपने को आवारा कुत्तों से घिरा हुआ बछड़ा सा महसूस करता है और मुर्छित हो जाता है। इस तरह के अति अमानवीय व्यवस्था-तंत्र के प्रतिकार में शैलेश मटियानी का कहना है – " मैं यह नहीं कहता कि एक आदमी को किसी दूसरे आदमी का जेब काटने, चोरी करने या गुंडागिरी फैलाने की छूट होनी चाहिए ; मगर मैं यह जरुर कहना चाहता हूँ कि जो सरकार अपने लाखों नागरिकों के लिए रोजी-रोटी की व्यवस्था करने में अपने को निकम्मा पाती हो, उसे ऐसे किसी भी कानून को बनाने का अधिकार नहीं है जो भुखमरी और बेकारी से मजबूर इंसान को रोजी-रोटी देने की जगह नृशंस यंत्रणाएँ देते हों।"4

'मिट्टी', 'प्यास' की तरह जिन्दगी की तलछट में एकदम गहरे धँसे मटियानी की एक और हृदयबेधक कहानी है 'भय'। इस कहानी में जिंदगी की मजबूर शक्ल को बचाये रखने की चिंता इतनी बड़ी हो जाती है कि उसके आगे नैतिक-अनैतिक,

पाप-पुण्य जैसे सवाल खोखले और बेमानी नजर आने लगते हैं । जहाँ जिन्दगी का सबसे बड़ा भय कोई भूत या दानव नहीं बल्कि आनेवाला कल है, जो अपने साथ भूख की दहशत लिए लगातार पास खिसकता आ रहा है । इसलिए तथाकथित अपराधी समझे जानेवाले कथावाचक की जेब में पड़ा चाकू कोई हथियार नहीं, बल्कि भूख और भय से लड़ने का औजार अधिक है । यही मजबूरी ननकू की घरवाली को एक लावारिस लाश(सीताराम) के पास बैठकर करुणा बटोरने का नाटक करने को उकसाती है । लास के पास बैठे, जोर-जोर से विलाप करती स्त्री को लोग उसकी विधवा समझकर पैसे दे जाते हैं और नाटक खत्म होते ही ननकू की घरवाली लाश पर से पैसे और सीताराम पर ओढ़ाई चादर उठाकर चल देती है । इस अमानवीय कृत्य को दर्शाते हुए मटियानी जी कहते हैं- " सीताराम की लाश पर से चादर हटा लेने के बाद, चुपचाप भाग जाने में दहशत होने का ख्याल उसे दबोच रहा हो- ऐसा भी नहीं है । "5

'इब्बूमलंग' मुंबई के कूड़े के ढेर में गजालत की जिंदगी बितानेवाले इबादत हुसैन का इब्बूमलंग(छद्मी फकीर) बनने की कहानी है और साथ ही हृदय की पुकार पर छद्म फकीरी का चोला उतारते इब्बूमलंग का फिर से इबादत हुसैन बनने की कहानी भी । कहानीकार ने इस कहानी में इबादत हुसैन का इब्बूमलंग(छद्मी फकीर) बनने की प्रक्रिया में शामिल प्रत्येक शक्ति का शिनाख्त करने की कोशिश की है । होटल के जुठे पत्तलों को धोकर मेहनताने में मिलनेवाले चरस की गोलियाँ खाकर दिन बितानेवाले इबादत हुसैन का इस्तेमाल भी धर्म के ठेकेदार(अपराधी नागप्पा) किस बखूबी अंदाज से करते हैं; इसका जीवंत दस्तावेज है यह कहानी । दरअसल इस बात को झुठलाया नहीं जा सकता है कि मुंबई जैसे महानगरों में केवल छद्मी बाबा ही नहीं बल्कि अपराध जगत से जुड़े लोग भी पैसा कमाने के लिए धर्म की ठेकेदारी करने से भी नहीं चुकते हैं । उन्हें यह अच्छी तरह से पता होता है कि यह आसानी से लोगों को मुर्ख बनाकर पैसा कमाने का सबसे सहज धंधा है । इसलिए वे महानगरों की इन झोपड़पट्टियों में इबादत हुसैन जैसे लोगों को ढूँढ़कर उसके मुँह से निकली गालियों एवं उसकी अश्लील हरकतों को सट्टे के नंबरों के साथ जोड़कर अपनी साधने में लगे रहते हैं । कहानीकार ने इस कहानी में वक्त के थपेड़ों से मार खाकर लानत की जिंदगी जीनेवालों को घोर अमानवीय कृत्य करनेवाले मशीनों में तब्दील करनेवाली अति चालाक शक्तियों की शिनाख्त करने के बरक्स यह भी बलाने की कोशिश की है कि भले ही ऐसे पात्र धर्म के ठेकेदारों द्वारा बनाये जाल में कुछ समय के लिए फँस जाते हैं पर बाद में इनमें से इबादत हुसैन जैसे कुछ लोग इससे बाहर निकल भी आते हैं क्योंकि उसके अंदर की मानवीय भावना पुरी तरह सुख नहीं जाती है ।

'दो दुखों का एक सुख' शरीर से अशक्त तीन भिखारियों – मिरदुला कानी, अंधा सूरदास और कोढ़ी करमिया के जीवन संघर्ष की अद्भूत कहानी है । यह एक ओर दारुण अभाव की जिंदगी जी रहे इन पात्रों की अद्भूत जिजीविषा की कहानी है तो दूसरी ओर अपने प्रेमी(अंधा सूरदास) के प्राणों की रक्षा के बरक्स अपनी देह तक से समझौता करती बेबस और लाचार मिरदुला कानी की कहानी भी है । इस बात को नकारा नहीं जा सकता है कि जीवन के तमाम अभावों में भी इतनी ताकत नहीं होती है कि प्रेम की भावना को दबा दें । कहानी के पात्रों की भी यही स्थिति है । उनमें प्रेम त्रिकोण बना हुआ है । अंधा सूरदास और कोढ़ी करमिया दोनों मिरदुला कानी को पाना चाहता है । अंधा सूरदास और कोढ़ी करमिया में झगड़ा हो जाता है और अंधा सूरदास को इस झगड़े का खामियाजा यह भुगतना पड़ता है कि उसे कोढ़ी करमिया के घर से बेघर होना पड़ता है । पर जब कोढ़ी करमिया को इस बात की जानकारी मिलती है कि जगत मिस्त्री सूरदास व मिरदुला को आश्रय देने के नाम पर उन्हें अमानवीय यंत्रणाएँ देता है तो उसे काफी ग्लानि होती है और वह उन्हें पुनः आश्रय देता है । पति सहित आश्रय पाने के एवज में मिरदुला को जगत मिस्त्री के अमानवीय यंत्रणा की तुलना में करमिया से अपनी देह का समझौता करना सह्य लगता है और वह सूरदास और करमिया के बीच नदी सी बह जाती है । बहने के क्रम में वह माँ बन जाती है । उस घोर अंधियारे की जिंदगी में एक संभावनामय नये जीवन को विकसित होते देखने की लालसा सूरदास और करमिया के हृदय को मिला देता है । कुल मिलाकर अंत में कहानी मनुष्य की लालसाओं एवं दुर्बलताओं का चित्रण करनेवाली एक अद्भुत संवेदनात्मक तीव्रता की कहानी बन जाती है । यही कारण है कि प्रकाश मनु इस कहानी को हिंदी साहित्य कालजयी कहानी मानते हुए कहते हैं – " 'दो दुखों का एक सुख' न सिर्फ मटियानी की कालजयी कहानी है, बल्कि यह हिंदी की उन शिखरस्थ कहानियों में से है जिनसे इनकी शक्ति और उँचाई को नापा जा सकता है । सच तो यह है कि अगर मटियानी ने सिर्फ यही एक कहानी लिखी होती तथा कुछ और न लिखा होता, तो भी वे इतने बड़े कहानीकार होते कि इनकी चर्चा के बगैर ह्दी कहानी का इतिहास नहीं लिखा जा सकता था । "6

मटियानी की 'एक कोप चा : दो खारी बिस्किट' मुंबई की फुटपाथी जीवन पर लिखित एक अद्भूत कहानी है । यह दो फूटपाथी प्रेमीयुगल रमन्ना और नसीम की कहानी है । कहानी की सबसे बड़ी खासियत यह है कि पुरी कहानी की पृष्ठभूमि ही मुंबई नहानगर के हाशिये में पड़ी भूखमरी, बेकारी व गंदगी की बजबजाती अंधेरी दुनिया है, जिसमें रमन्ना और नसीम की जिंदगी जुगनु की तरह जलती-बुझती दिखाई पड़ती है । पर जब कहानी में नसीम का प्रसंग आता है तो वहाँ कहानी रोंगटे खड़े कर देनेवाला बन उठता है क्योंकि वहाँ हमें नसीम(फूटपाथी औरत) अपनी पेट

की भूख मिटाने के लिए एक कप चाय व दो खारी बिस्किट के बरक्स अपने जिस्म का सौदा तक करने की मजबूरी और दुःख को व्यक्त करने के लिए रमन्ना से कहती हुई मिलती है - " हम औरत लोगों की जिंदगी भी क्या बदनसीब...मैं भी कैसी बेशरम हूँ ? रोटी के लिए बोसे भी देना और बोटियाँ भी...थू है ऐसी जिंदगी पर । "7 वास्तव में मटियानी जी ने कहानी के इस चित्र की प्रस्तुति के द्वारा हमारे तथाकथित विकसित समाज के मुँह पर करारा थप्पड़ मारकर मानवीयता की भावना को जगाने के बरक्स पाठकों के हृदय को झकझोरते हुए लज्जित करने का ही काम किया है क्योंकि उनके अनुसार जिस समाज में अपनी पेट की क्षुधा मिटाने के लिए एक गरीब और मजबूर औरत को महज एक कप चाय व दो बिस्किट के भरक्स अपनी देह का सौदा करना पड़े, उसे स्वयं को विकसित कहने का भोंडा मजाक तो नहीं ही करना चाहिए । मटियानी जी को इस नारकीय स्थति को मिटाने को रास्ता संघर्ष को ही मानते हैं । मटियानी जी को विश्वास है कि इस फूटपाथी नारकीय स्थिति को बदलने का बीड़ा स्वयं इसी वर्ग को ही उठाना पड़ेगा क्योंकि उच्च वर्ग को इससे कोई लेना-देना न था, न है और न रहेगा ही । यही कारण है कि मटियानी जी ने कथा के अंत में रमन्ना को नसीम को हवस का शिकार वनने से बचाने के लिए वर्दीधारी हवलदार का गला दबोचते दर्शाया है । यास्तव में देखा जाये तो कहानी को पढ़ते हुए यहाँ आकर हर पाठक की मनःस्थिति ही हवलदार का गला दबोचनेवाली ही बन जाती है; जो दरअसल हमारी सामाजिक व्यवस्था और सरकारी नीतियों का गला दबोचने की इच्छा को ही बयां करती नजर आती है ।

मटियानी की 'रहमतुल्ला' कहानी में मैली जिंदगी की विडम्बना के साथ-साथ धार्मिक विडम्बनाएँ भी घुल-मिल गई है । यह एक अनाथ लड़के की कहानी है जो अक्सर मस्जिद की सीढ़ियों पर बैठा दिखाई देता है । वह एकदम मैला, गंदा, भौंडा दिखनेवाला अनाथ लड़का है । उसके साथ धर्म के पचड़े, गरीबी और एकाध चमत्कारिक घटना जुड़कर शुभ-अशुभ का ऐसा तालमेल बना देती है कि लोग उससे दूर रहने में ही भला समझते हैं । रहमतुल्ला के पिता मुसलमान व माँ हिन्दू थी । इसलिए वह कभी हिन्दू तो कभी मुसलमान की ओर खिसकता दिखाई देता है । वास्तव में हिंदू-मुसलमान प्रसंग के मार्फत इस सच्चाई से पाठकों को अवगत करवाया है कि गरीबी इंसान का इतना बड़ा अभिशाप है, जिससे हिन्दू या मुसलमान सभी को बदबू आती है । यह कहानी हिन्दू और मुस्लिम धर्म के(मानवीयता के) स्वनामधन्य ठेकेदारों के माथे पर सवालिया निशान लगाती प्रतित होती है ।

निष्कर्षत: हम कह सकते हैं कि शैलेश मटियानी की कहानियों में मुम्बई नगर के हाशिये का समाज अपने बजबजाते यथार्थ के साथ चित्रित हुआ है । इस समाज से जुड़ी इनकी कहानियों की सबसे बड़ी विशेषता यह है कि इनमें लेखक का अन्य

लेखकों की तरह सहानुभूतिपूर्ण रवैया के बजाय स्वानुभूतिपूर्ण रवैया ही सर्वत्र उद्भासित होता नजर आता है । कहने का तात्पर्य है कि इनकी कहानियों मे सर्वत्र भोगा हुआ यथार्थ ही दिखाई पड़ता है । यह कहना समीचीन होगा कि इनकी कहानियों में व्यक्त सामाजिक जीवन का यथार्थ भारतीय समाजिक-व्यवस्था एवं सरकारी नीतियों पर कुठाराघात करता है तथा समाज के उपेक्षित वर्ग, हाशिये पर पड़े वर्ग व परम्परागत पुरुष मानसिकता के शोषण तले दबी स्त्री वर्ग के अंतिम इकाई तक को लड़ने की शक्ति प्रदान करता है । यही कारण है कि प्रकाश मनु इन्हें हाशिये के लोगों को प्यार करनेवाला हिंदी का सबसे बड़ा लेखक मानते हैं – " सच तो यह है कि दलितों व निचले वर्ग के लोगों से प्यार करनेवाला उनसे बड़ा कोई और लेखक हमारे बीच हुआ ही नहीं । "8

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Emergence, entry and guarding behaviour at the nest-opening site of the yellow crazy ant *Anoplolepis gracilipes* (Jerdon)

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Abstract
The yellow crazy ant *Anoplolepis gracilipes* is a notorious invasive animal species causing serious problems in the areas where it has been introduced so far. Accordingly various attempts have been made to collect biological information to manage the said ant species. Recently, we had the opportunity to study the behavioural events in respect to emergence from the nest, entering the nest as well as guarding the nest –hole in *A. gracilipes* occurring in the Achhruram Memorial College campus, Purulia, West Bengal, India. It is revealed that these ants are habituated to go out of the nest on average 13.92 ± 1.12 SE, entering the nest 10.9 ± 0.75 SE and guarding the nest –hole 12.73 ± 1.16 SE (N=85) per minute during day hours. While such numbers differed significantly ($p < 0.05$) with respect to behavioural events considered for studies, no significant difference could be established in respect to seasons ($p < 0.06$). Thus the present findings suggest that, being invasive species *A. gracilipes* have developed various devices to protect them from the attack of endemic species on way of exploiting the endemic species.

Keywords: *Anoplolepis gracilipes* ants, emergence, entry, nest guarding

Introduction
The yellow crazy ant *Anoplolepis gracilipes* is one of the notable invasive animal species as could be revealed from the listed one hundred species presented by Lowe *et al* (2004) [10]. The said ant species is a native of the moist tropical lowlands of Southeast Asia and adjacent islands of the Indian and Pacific oceans (Guénard, 2019) [4]. However, nowadays, these ants are creating problems as invasive agents in certain parts of Australia, New Zealand, Chile, Durban, South Africa, Zayul, Tibet, Mexico, as well as in the Caribbean, Central and South America (Küchler, 1964, Lester and Tavite, 2004, Wettrer, 2005) [7, 9]. Almost in all regions *A. gracilipes* are causing serious problems on way of threatening the endemic species of the introduced localities and they are very much involved in biodiversity degradation. (O' Dowd *et al*. 2003, Lester and Tavite, 2004, Drescher *et al*. 2007, Plentovich *et al*. 2018) [14, 9, 3, 15].

Following invasion and with the development of subsequent environmental hazards various workers have paid due attention to note the biology and inter-specific interactions of *A. gracilipes* in respect to the ecosystem where these ants became serious nuisance (Lester and Tavite, 2004, Abbott, 2005, Drescher *et al*. 2007, Kaiser - Bunbury *et al*. 2014, Lee *et al*. 2017, Plentovich *et al*. 2018) [9, 1, 3, 5, 8, 15]. However, still to date no attention has been paid by any worker to note the behaviour of these ants in respect to emergence from the nest, entry into the nest from the outside and guarding strategy encircling the opening of the nest. Thus, in course of our studies on the bioecology of ants occurring in and around Jhalda (Achhruram Memorial College premises), Purulia, West Bengal, India we took the liberty to note these behavioural events during the period of day hours at an interval of 1 hour, and the findings are worth reporting.

Materials and Methods
The nest we considered for our studies was located on the ground but very close to a big *Albizia lebbeck* tree. The ants were seen to come out of the nest through a hole as well as entering into the nest through the said hole of and on, while certain individuals were seen to roam encircling the nest hole all along. We spent 1 minute in each occasion during any time in an hour between 06.00 h and 18.00 h at random during the period of September 6, 2008 and December 23, 2014. The data collected during an hour period were considered to calculate the mean and standard error values. Also two-way ANOVA was applied to justify the effect of the hours of the day in respect to the strategies developed by the ants regarding the frequency of emergence from the nest and entry into the nest keeping the number of ant individuals engaged in guarding the nest-hole in view.

Results
During the period of six years, we recorded data on 85 days, irrespective of seasons, at random, on the frequency of emergence from the nest, entry into the nest as well as the numbers of *A. gracilipes* were guarding the nest-hole. The behavioural variations in respect to these events have been shown in Figs. 1-12. Irrespective of study hours 1-64 (12.73 ± 1.16 SE) ant individuals kept them engaged in nest guarding, 0-33 (10.9 ± 0.75 SE) were entering the nest, and 1-59 (13.92 ± 1.12 SE) were emerging out of the nest.

Results of ANOVA tests clearly indicate that the number of ant individuals took part in executing the behavioural events like emergence from the nest, entering the nest and guarding the nest –hole differed significantly ($p < 0.05$) throughout while such numbers irrespective of seasons did not differ significantly ($p < 0.06$).

Explanation of Figures

Figs. 1-12 represent the mean (+/- SE) number of ants noted guarding the nest-hole (S), entering the nest (I) and emerging out of the nest (O) during one minute time period between 06:00 h and 07:00 h

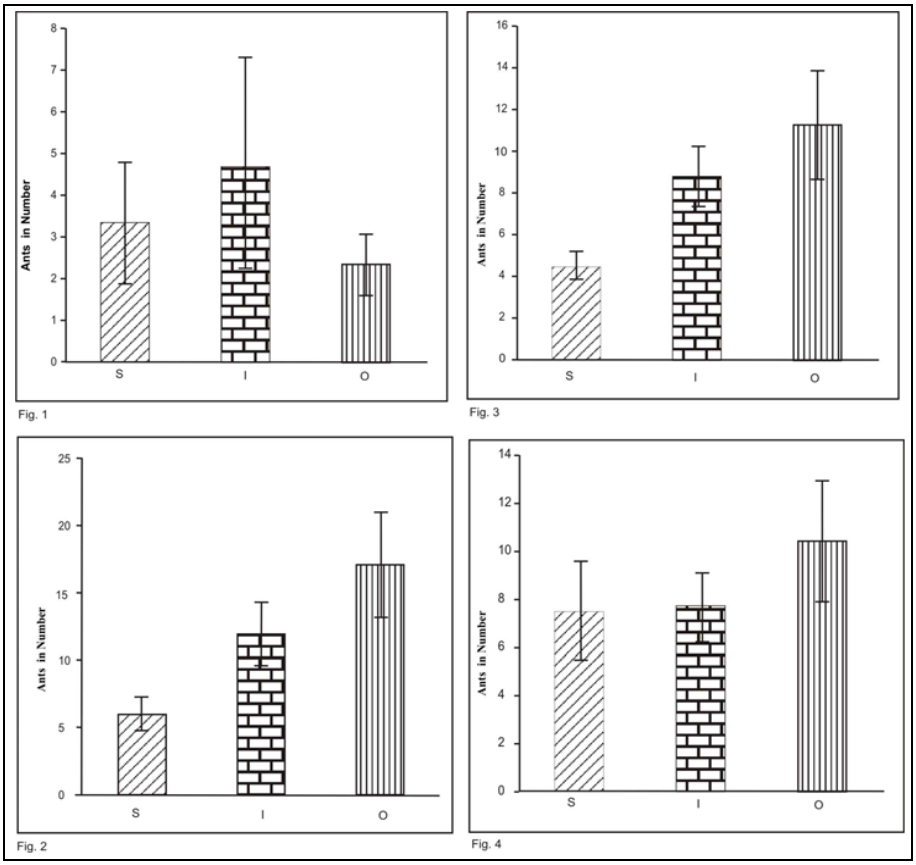

Fig 1: 07:00 h and 08:00 h (Fig.2), 08:00 h and 09:00 h (Fig.3), 09:00 h and 10:00 h (Fig.4), 10:00 h and 11:00 h

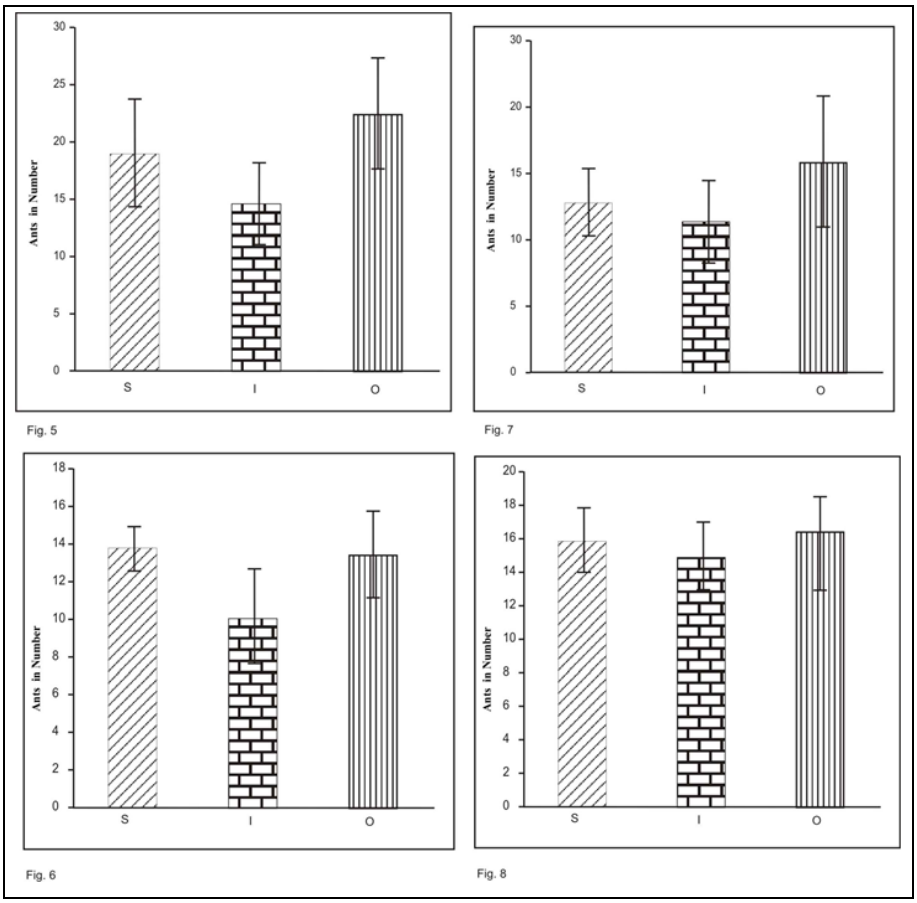

Fig 5: 11:00 h and 12:00 h (Fig.6), 12:00 h and 13:00 h (Fig.7), 13:00 h and 14:00 h (Fig.8), 14:00 h and 15:00 h

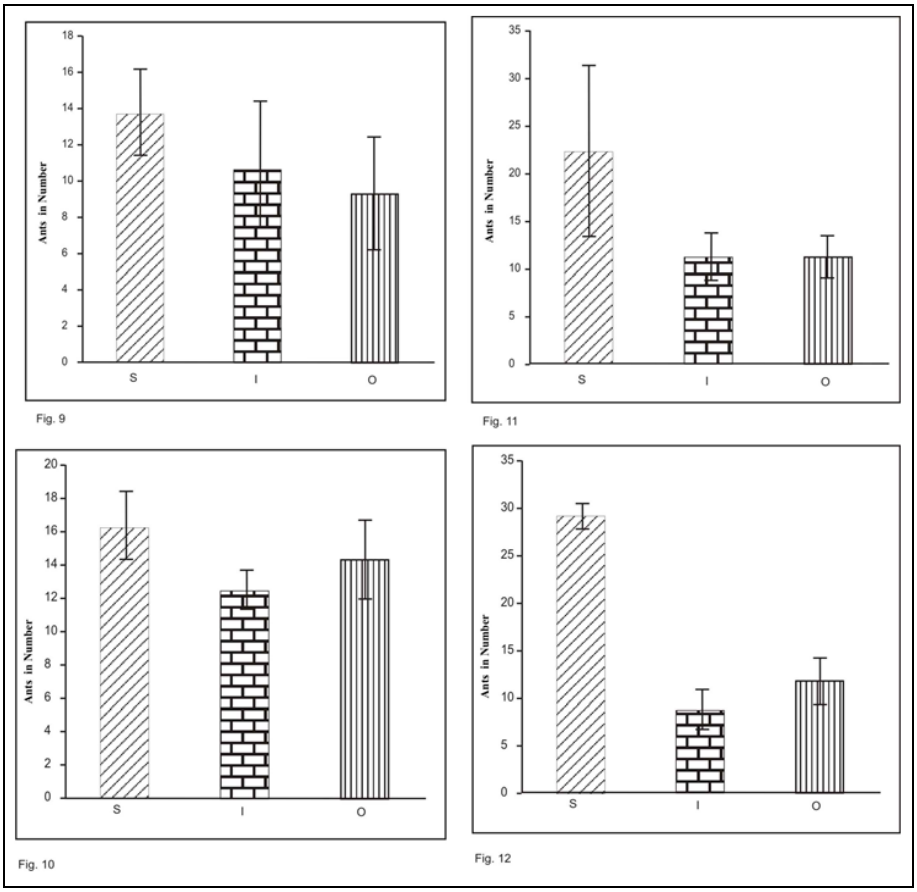

Fig 9: (Fig.9), 15:00 h and 16:00 h (Fig. 10), 16:00 h and 17:00 h (Fig. 11), 17:00 h and 18:00 h (Fig.12).

Discussion

From the results it is clear that the yellow crazy ants *A. gracilipes* are habituated to maintain a strategy for effective foraging efforts at least in respect to the hours of the day time. Though it is customary that the foragers go out of the nest from time to time and also entry of the individuals is continued simultaneously the occurrence of certain number of individuals encircling the opening of the nest is undoubtedly, a matter of interest. *A. gracilipes* ants have been listed as one of the world's most invasive alien species (Lowe. *et al*. 2004) [10]. It is reported that *A. gracilipes* are apt to establish them in a new geographical area because of traits such as aggression toward other ant species, little aggression toward members of their own species (Kirschenbaum and Grace 2008) [6]. These ants get much of their food requirements from scale insects, aphids and other Sternorrhyncha. Also, they get their carbohydrates from plant nectar. Since report on the food storage of these ants is not on record, it is, at this moment very difficult to explain why certain members remain engaged at the nest-door as guards. It may assume that, there exists possibility of robbing food from the nest by other ant species or by the members of the same species belong to the other nest-colony.

Food-snatching, robbing of the food materials from the nest as well as cleptobiosis habits have been observed in the ants *Pheidole roberti, Paratrechina longicornis, Oecophylla smaragnida, Tetraponera rufonigra, Messor aciculatus, Prenolepis imparis, Ectatomma recidumand,* and *Messor capitatus* (Lynch *et al*., 1980, Yamaguchi, 1995, Breed *et al*., 2012, Naskar and Raut 2019) [11, 17, 2, 12]. Also, in respect to such behavioural events fighting between the owner and snatcher ants have been noted in some ant species (Naskar and Raut 2020) [13]. Thus, it is most likely that, *A. gracilipes*, being an invasive species creates different kinds of conflicts at least with endemic ones to establish their aggressive and dominant habit so as to keep the victimized ant species under fear psychosis. This sort of behaviour may force the endemic species for retaliation, if possible unitedly. Perhaps, anticipating such an attack *A. gracilipes* left no effective strategy to protect them and to establish them in a new area successfully.

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Taxonomic analysis of some forest insects used in the diets in Mexican rural areas: evaluation and perspectives

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RESEARCH ARTICLE

Abstract

In the present work a taxonomic analysis of Mexican edible forest insects along with their host plants have been reviewed. We have recorded 73 insect species under 26 families and 6 orders, namely: Orthoptera, Hemiptera-Heteroptera, Lepidoptera, Coleoptera, Diptera and Hymenoptera, with the highest number of species belonging to Coleoptera (19) followed by Hymenoptera (18). Additionally a total of 51 host plant species under 17 families, and their distribution among the different vegetation types also have been presented. The importance of insects in the diet of rural communities has been discussed in the light of different socioeconomic and biological factors. These issues must be addressed and resolved, to carry out the coherent management of edible insects, considering the traditional knowledge that the rural inhabitants possess.

Keywords: aboriginal, entomophagy, Mexico

1. Introduction

Human societies have consumed insects for thousands of years as emergency foodstuff, staple foodstuff or delicacy. At a global level, in total 1,900 edible insect species have been registered until today (Van Huis *et al.*, 2013). This proves entomophagy (the habit of consuming insects by man) has persisted for centuries, being insects a highly nutritious food resource (Ramos-Elorduy, 2004). Unfortunately, serious scientific works have started at a comprehensive level only in the last decade (Dossey *et al.*, 2016; Halloran *et al.*, 2018; Schowalter, 2013; Van Huis and Tomberlin, 2017).

In relation to their abundance, even though insects represent the largest quantity of biomass in forests, they have been little studied in these ecosystems. In addition, these animals have an enormous economic value due to the environmental services they render. They are main pollinators, they carry out diverse cleansing activities that include manure elimination, carcass decay and organic matter decomposition and recycling, they are soil producers and conditioners, and a great food resource for wildlife (invertebrates, amphibia, reptiles, birds, fish and mammals) (Govorushko, 2018). In spite of all this, almost all the forest insects are considered as pests by the forest engineers (Cibrian *et al.*, 1995). In Mexico, indigenous people have vast entomophagic traditional knowledge on various forest insects, and hence the great diversity of forest habitats and the variety of edible insects represent a range of opportunities for its management and exploitation.

Studies on forest insect collection and management are limited in Mexico. However, there are successful examples in other parts of the world that illustrate the potential of forest insects as foodstuffs for man, as in case of the larvae of the weevil *Rhynchophorus ferrugineus papuanus* (Coleoptera: Curculionidae), called sagu parasites or sagu worms of the palm *Metroxylon sagu* (Family: Arecaceae); in Papua New Guinea they are an important nutritious food resource (Onyeike *et al.*, 2005). This is also the case of the palm weevil *Rhynchophorus phoenicis* in Cameroon (Fogot *et al.*, 2015). The rustic exploitation of the edible

caterpillars *Gynanisa maya* and *Gonimbrasia zambesina* (Lepidoptera: Saturniidae) is made by the natives in the north of Zambia and the Democratic Republic of Congo, as they frequently bring young larvae from the forest and put them on acacias (Family: Fabaceae) near their homes and rear them until they are ready to be eaten. Finally, in the tropical areas of America toasted larvae and adults of the palm weevil *Rhynchophorus* spp., are harvested from different palm species and are consumed as delicacies.

Insects are highly nutritious in terms of protein and amino acids (Ramos-Elorduy *et al.*, 1997) and in the Mexican Republic they are traditional foodstuffs in the rural areas, they are used by diverse cultural groups such as: *chol*, *huasteco*, *lacandón*, *maya*, *mazateco*, *mazahua*, *mixteco*, *nahuatl*, *otomí*, *otopame*, *popolaca*, *tarahumara*, *tarasco*, *tlapaneco*, *tojolabal*, *totonaco*, *tzeltal*, *tzotzil*, *zapoteco*, *zoque* and *mestizos*; as hundreds of species have been consumed since pre-Hispanic times (Ramos-Elorduy, 2004; Ramos-Elorduy and Pino, 1989, 2002; Ramos-Elorduy *et al.*, 1985, 1998b, 2002a), more in depth studies on forest insects are of great importance. On the other hand, the Mexican flora is one of the most diverse on a worldwide level. In Mexico the concept of forest vegetation is defined as the group of plants and fungi that grow and develop naturally, forming forests, rainforests, arid and semiarid zones and other ecosystems, giving place to the development and well-balanced coexistence of other natural resources and processes.

The vegetation types most widely distributed in the country are the following ones:

- Coniferous forest: vegetation dominated by evergreen trees of the conifer group among them pines (*Pinus*) and firs (*Abies*) are the dominant ones. They are generally found in the temperate and cold climates of the upper zones of mountain ranges that exist almost in the entire country.
- Oak forests: are a plant community made up of different oak species of the genus *Quercus* that grow in diverse ecological conditions, ranging from sea level to almost 3,000 m altitude. The distribution of oak forests is similar to the coniferous forests.
- Mountain cloud forest: in terms of physiognomy it is a dense type of vegetation, typical of mountainsides that are protected from strong winds and excessive sunshine. They develop at an altitude where fog banks appear and where mist forms throughout the year. This is an exuberant forest, rich in ferns and lianas, as well as epiphytes that grow on the trees. An important portion of the flora is endemic. These forests are found along the Sierra Madre Oriental where they extend along certain portions of several states, usually on mountainsides facing the Gulf of Mexico.
- Evergreen rainforest: the vegetation is dominated by several tree species, it is one of the most diverse biological communities in the world, it is found in warm and rainy climates. The crown of the trees can be higher than 40 m and conserves an important part of its foliage throughout the year. It is found in San Luis Potosí, Veracruz, and certain regions of Hidalgo, Puebla, Oaxaca, Chiapas Tabasco, Campeche, Quintana Roo, Yucatán, Nayarit and Guerrero.
- Deciduous and sub-deciduous rainforests: the vegetation is dominated by different tree species whose leaves are deciduous. They develop in warm environments with summer rains, they are divided in medium and low according to the height of the dominant arboreal vegetation. The canopy rarely surpasses 15 m in height, even though in some cases it can reach up to 30 m. Among the characteristic genera are *Bursera* sp. (Family: Burseraceae) and *Ceiba* sp. (Family: Malvaceae) and several species of columnar cacti. It exists, for example, in a discontinuous form, from the centre of Sinaloa to the coastal zone of Chiapas, Yucatán and Veracruz.
- Thorn forest: it is a community dominated by thorny trees such as ebony, *cascalote*, *brasil* and *mezquite*. It covers a great extension in the north-western coastal plains, from Sonora to Sinaloa, the Balsas Depression and the Isthmus of Tehuantepec. Along the Gulf of Mexico coast it covers a big area in Tamaulipas, Campeche, Quintana Roo, Chiapas and Yucatán.
- Cactus scrub desert: vegetation dominated by shrubs, typical of arid and semiarid zones, this type is the most widespread in Mexico. It covers the greater part of the territory of the Baja California peninsula, as well as great extensions of the coastal plain and the low mountains of Sonora. It is typical of great areas in the Central Basin from Chihuahua and Coahuila to San Luis Potosí, Guanajuato, Hidalgo, Estado de México, Puebla, Oaxaca, Coahuila and Tamaulipas.
- Natural grasslands: it is a community dominated by grass species, occasionally accompanied by herbaceous species and shrubs of different families. It is found in Sonora and even though it exists in almost all the states of the country, it is most widely distributed in Chihuahua, Jalisco and Guanajuato.
- Mezquite forest: the plant community is dominated mainly by *mezquites* (*Prosopis* spp.) that develop as shrubs. It is common to find this community in a mixed state, for example, with the *huizaches* (*Acacia* spp.), both from the Family Fabaceae. It is distributed mainly in the central plateau, Baja California Sur, Sonora, Tamaulipas and Jalisco.

The forest vegetation of arid zones is the plant community that develops in a spontaneous way, in the regions with arid or semiarid climates, forming masses greater than 1,500 square meters (CONAFOR, 2012).

Considering the aforementioned entomological and botanical aspects and in face of the lack of entomophagic

information about forested areas, we have carried out this research with the objective of gaining knowledge about the edible forest insects of Mexico, as well as of their hosts, and thus analysing and discussing the perspectives for their tangible exploitation.

2. Materials and methods

The Institute of Biology of the National Autonomous University of Mexico (UNAM) is carrying out research in the field of 'insects as future protein sources' since 1976 (Ramos-Elorduy, 1982). Studies have been conducted in several places of the Mexican Republic like the Milpa Alta municipality of Mexico City (Ramos-Elorduy *et al.*, 1992), and in the states of Chiapas (Ramos-Elorduy and Pino, 2002), Guerrero (Ramos-Elorduy *et al.*, 1985), Hidalgo (Ramos-Elorduy and Pino, 2001a), Estado de México (Ramos-Elorduy *et al.*, 1998a), Oaxaca (Ramos-Elorduy *et al.*, 1997), Puebla (Ramos-Elorduy *et al.*, 1988a) during last 4-5 decades by means of a series of research projects that comprised field work, data collection through questionnaires, and collection of both insects and of their host plants, many of which were still unknown. The insects that were collected in diverse plant communities were placed in jars with 70% alcohol and the plants were preserved in a botanical press; in both cases we have recorded the collection data that include date, locality, collector's name, common name, native linguistic name, vegetation type and edible stage of development. The collected specimens were taken to the Institute of Biology at UNAM to be mounted, labelled, catalogued and preserved for future use. For the taxonomic work we have collaborated with several botanists and especially entomologists, as the identification of immature stages such as larvae, pupae and nymphs of different insect orders is extremely difficult. The identified insects are kept in the National Collection of Edible Insects which is housed in the Department of Zoology, UNAM.

We have also carried out a retrospective bibliographical review that consisted of diverse sources such as thesis, research papers and books on entomology where edible forest insects have been reported; in this regard the works of Durst and Shono (2012), Johnson (2012), Martínez (2016), SEMARNAT (2010) and Rzedowski (2006) were found to be extremely useful.

3. Results

The edible insects collected in the forest vegetation of Mexico are depicted in Supplementary Table S1, that includes order, family, scientific name, common name, edible stage of development, consumption site and the related references. To date, we have registered 73 species of edible forest insects under the orders Orthoptera (11 species, i.e. 15.06%), Hemiptera-Heteroptera (10 species, i.e. 13.69%), Coleoptera (19 species, i.e. 26.02%), Lepidoptera (12 species, i.e. 16.93%), Diptera (3 species, i.e. 4.10%) and Hymenoptera (18 species, i.e. 24.65%). The most represented orders are Coleoptera (genus *Mallodon* being the dominant one with 4 species that are commonly known as 'stick worms'), and Hymenoptera that includes social insects, that is, bees, wasps and ants. The most demanded and commercialised insects are grasshoppers, maguey red and white worm, *escamoles*, *jumiles*, a type of ants known as *chicatanas* and the cochineal grana; the latter has multiple applications in pharmaceutical, food, cosmetic and wine industries. We therefore appreciate that the great diversity of forest habitats harbouring edible insects presents an array of opportunities for innovative management of edible insects so as to simultaneously contribute to maintaining habitat diversity for other life forms (Defoliart, 1997). Nevertheless it is necessary to assess the link between insect gathering and the forest ecosystem, wildlife conservation and bushmeat consumption patterns (Vantomme *et al.*, 2004).

Some examples of case studies are:

Cuetla (*Arsenura armida*) – *jonote* (*Heliocarpus* sp., Family: Tiliaceae): in a study carried out in the Sierra de Zongolica, Veracruz, the Sierra Negra of Puebla and the Sierra Norte of Chiapas (Gomez, 2009), 63 individuals were interviewed, 84% (53.7 persons) of which are collectors that have learned the activity throughout generations for more than 30 years, while the rest (9.3) are collector-vendors of these larvae. Collection season begins in June and is over by November, and it is closely related to the rainy season, it is carried out in the woods where there are approximately 15 *jonote* trees per hectare, 8 of which are covered with worms each season. Collection is carried out only one day and in only three trees as a conservation strategy, and it is done when the worms form groups in the trunk and branches, and only the most 'fat' are collected. For collection a container is used (plastic bags, buckets, satchels), and they are fetched using poles or people climb the trees to collect them by hand. Per tree and per day a mean of 17.2 kg is collected, 76% of which is for self-consumption, 16.5% is given as a present to relatives and 7.2% is sold. A management practice can also be understood as an incipient breeding technique or 'protoculture' and it consists on moving *cuetlas* of 5 or 6 cm in length that are on trees far from the homes to *jonotes* that are nearer, so that their development can be monitored until they are 'fat' enough, thus guaranteeing their production and consumption; this practice is implemented to obtain a higher production in less time, thus rendering benefits for the local population just as, for example, the 'farm' culture carried out in Cameroon for the larvae of the weevil *Rhynchophorus phoenicis* (Fogot *et al.*, 2015), that are considered a veritable delicacy (Dounias, 2010). Of the interviewed persons, 98.8 % like to eat worms, they say they taste like *chicharrón*, pork meat or fish, pumpkin seeds or

sheep barbecue; 92.0 % of the families consume a mean of 8.2 kg per season, 62.15% of the interviewed persons point out that consumption has been steady and is on the rise. For their culinary preparation they are cleaned (the intestinal tract is taken out) and then they are washed, boiled and drained, and there are recipes to prepare them fried, in broths, toasted or roasted, with scrambled eggs, as pie filling, in stews, etc. People prefer them fried as they say they are easy to prepare and are an excellent appetiser. Of the interviewees, 95.4% say they are innocuous, and there are different forms in which they can be preserved such as sun dried, toasted, refrigerated in a plastic bag or refrigerated after being boiled, and so they can last for a whole year. Their commercialisation is not a priority as only 6.6 % of the interviewees, that is 4.1 persons, carry out this activity; nevertheless they point out that it is an important source of economic income during the season in which they are abundant and that they have been selling the product for 17.4 years. For their commercialisation they are boiled with salt and chili, they are cleaned and washed, and they are sold directly in the markets to retailers or to customers on demand. In the markets they are sold in 1 kg bags and in 200 g bags, in a small plate used as a measure for 100 g or boiled and wrapped in a *tamal* leaf, also in this case approximately 100 g (Gómez, 2009).

The *chichas* (*Mallodon dasystomus* (Say) and *Mallodon molarius* Bates 1879) – walnut tree (*Carya illinoinensis*, Family: Juglandaceae). Their collection takes place between January and April in the localities of Tlatepexe, Pedregal de Zaragoza and San Cristóbal in the region of Metztitlán, Hidalgo, mainly in fallen trunks of walnut trees (*Carya illinoinensis*) in different states of decay. For larvae collection wood is removed by means of an electric saw, a hatchet, wedges and entomological pliers so as to render its removal easy (Acosta *et al.*, 2019). Due to their saproxilophagous habit this larvae have diverse secondary hosts such as *Acer* sp. (Aceraceae), *Bursera simaruba* (Burseraceae), *Cordia inermis* (Boraginaceae), *Quercus* sp. (Fagaceae), *Inga* sp. (Mimosaceae), *Ficus* sp. (Moraceae), *Salix* sp. (Salicaceae) and *Celtis* sp. (Ulmaceae) (Maes *et al.*, 2010). In respect to their management, the larvae thus collected are placed in plastic boxes that contain the host's wood and sawdust at room temperature and their development is monitored daily till they reach the adequate size for their consumption and/or to obtain new adults so as to start over their biological cycle. For their preparation the biggest *chichas* are placed for 24 hours at room temperature in a plastic tray so that they pass excreta and then they are washed to eliminate organic detritus that sticks to their bodies. Afterwards they are prepared according to the consumers taste, specifically fried, toasted, covered in ground bread or as appetisers or garnish that are eaten accompanied by *tortillas* and sauce (Acosta *et al.*, 2019). The diverse larval phases are considered a complementary foodstuff in terms of diet and nutrition for the population of Metztitlán, as the majority of the inhabitants recognise their nutritive value in terms of proteins and minerals (Ramos-Elorduy, 1998b; Ramos-Elorduy and Pino, 1990) and above all because obtaining larvae does not have an economic cost. Collection is mainly for family self consumption, people consume them because they like them, because it is a tradition and because they are abundant, and they also say they are clean and tasty. In this case it is convenient to point out that insects play an important role in the functioning of the ecosystems they inhabit by means of a group of processes that can be classified as ecosystemic services, for example in the regulation of nutrient recycling when they contribute to the decay of wood. Therefore, in the broadest sense, insects have enormous economic value in terms of the ecological services they provide (Johnson, 2012). The relevance of dead wood has even been recognised, for example, for the biodiversity of forest systems as an important habitat for wildlife (Merganičová *et al.*, 2012) and as an indicator and key component of the forest structural diversity and functioning, carbon sources and fuel loads (Vandekerkhove *et al.*, 2009; Woodall and Williams, 2005) and is becoming an integral part of forest management as it performs a function very important for purposes of conservation and biodiversity management (Marage and Lemperiere, 2005). These insects are currently threatened by the expansion of agriculture, since in these localities the following are cultivated: corn, bean, lettuce, walnut, tomato and cauliflower.

Chicatanas (*Atta* spp.) – black *zapote* (*Dyospyros digyna*). The ants of the genus *Atta* have been used since pre-hispanic times as food and it is part of traditional nutritional habits because of their agreeable taste and its high protein content (Sahagún, 1975). In Huatusco, Veracruz, *Atta cephalotes* is the most common species and, as we have said, they are known as *chicatanas*, a term that applies to the reproductive caste especially in the localities of El Coyolito and Palmillas in the aforementioned municipality of Huatusco, Veracruz, where their nests are abundant (Landero *et al.*, 2005). Their collection is done in May and June (the highest biomass is present in June), when the ants defoliate two or three plants and place the leaves over the nest; this is a peculiar habit of the species that enables their capture by hand when they exit the nest for their nuptial flight. They can also be collected by placing a broom made out of dry sticks over the nest, then by awaiting for the ants to climb it and then shaking it over a bucket full of hot water so as to prevent their flight. In these localities two questionnaires were applied, the first to 100 persons of the municipality head of Huatusco, in the market and surrounding area, the sample consisting of 60 adults between 20 and 65 years and 40 children between 8 and 10 years. The second was applied to 50 adults between 20 and 65 years and 20 children between 8 and 10 in the village of El Coyolito, with a similar sample for Palmillas (Landero *et al.*, 2005). According to the replies of the adults of rural zones, 100% knows them, 100% consumes

them, 90% prefers them toasted, 10% prefers them in a sauce made of *pajarito chili* or they eat them raw and only 30% knows their price in the market which varies because when populations are high they are quite cheap but become expensive when populations are low. In relation to their consumption, of the children of rural areas interviewed, 100% knows them and consumes them toasted, of the adults of urban zones 90% knows them and 80% consumes them toasted and 20% in sauces, and 85% knows their price in the market. Of the children in urban zones 60% knows them and of them 100% consumes them, 50% toasted and 50% in the form of soft toffee. And as in rural zones only 30% knows their price, which means people collect them for self consumption, that is, they are not commercialised, some persons even freeze them to consume them along the year. According to the questionnaires their consumption is higher in rural zones where it contributes significantly to the diet's nutritional content; this is different from what happens in urban areas where alimentary biodiversity is higher as well as purchasing power, and so we can say this knowledge is being lost in a day to day basis, especially in the children that live in cities because of the introduction of junk food and the emigration of people from rural areas due to the lack of employment sources (Landero *et al.*, 2005). Nevertheless, presently there is a growing interest among entrepreneurs for manufacturing products based on *chicatanas* (Avendaño, 2002). In the same manner there are reports of great business opportunities when these insects are classified as 'exotic foods' and their culinary preparation becomes gourmet by the addition of diverse dressings that render them more attractive, palatable and colourful. For example, they are prepared in sauces known as 'saucettes', soft toffees, salts and bars known as *Nukuquetas* whose flavour is similar to that of nuts. All this is a sign of their gastronomic versatility and its potential in the national market; they are even asked for by many restaurant owners particularly in the USA and in some European cities such as London, Berlin, Barcelona, etc., where they are canned or covered in chocolate and included in cakes and cookies that are successfully sold in gourmet outlets.

In Supplementary Table S2, we summarise the taxonomical list of the host plants of the edible forest insect; it includes vegetation type, scientific names and the related references. We have documented a total of 52 forest host plants under 18 families. The ones that harbour the maximum number of edible hosts insects are Fagaceae (13 species), followed by Poaceae (11), Asparagaceae (8) and Pinaceae (7). The families we report and the genera they encompass are the following: Altingiaceae*: Liquidambar*, Arecaceaea: *Cocos*, Asparagaceae: *Agave*, Asteraceaea: *Thito*nia, Betulaceaea: *Alnus*, Cactaceae: *Opuntia*, Cupressaceae: *Juniperus*, Ebenacaea: Dyospyrus, Ericaceae: *Arbutus*, Fabacaeae: *Prosopis*, *Acacia* Fagaceae: *Quercus*, Juglandaceae: *Carya*, Poaceaea: *Bromus*, *Chloris*, *Bouteloua*, *Hilaria*, *Cynodon*, *Echinochloa*, *Andropogon*, *Paspalum*, *Panicum*, Solanaceaea: *Solanum* and Tilliaceae: *Heliocarpus*.

We have documented a total of 6 types of vegetation and the number of edible insect species pertaining to each one are: (a) savannah (12); (b) pine-oak forest (12), oak forest (7), pine forest (8); (c) rain forest (8); (d) tropical deciduous forest (8); (e) cloud forest (4); and (f) cactus scrub desert (16). In the vegetation types known as pine-oak forest (oak forest and pine forest), cactus scrub desert and savannah, we registered the highest number of edible insect species, being 27, 16 and 12 (Figure 1 and Table 1) (SEMARNAT, 2010). In the case of the pine-oak forest, this result is due to the high number of genera and species that make it up and for the case of cactus scrub desert the explanation may lie in the fact that it is the most prevalent vegetation type in Mexico.

It is important to point out that in Supplementary Table S2 we only include the hosts of the localities where the insects reported in Supplementary Table S1 were collected; because of this, the number of host species may vary in a significant manner. For example, if we consider the ecologic and geographic assets of the *escamoles* (*Liometopum apiculatum*), we can see they are found in all ecosystems, ranging from deserts to forests (Hernández *et al.*, 2017) while the *cuetla* (*Arsenura armida armida*) has been registered for the states of Chiapas, Colima, Estado de México, Nuevo León, Oaxaca, Puebla, Quintana Roo, San Luis Potosí, Tabasco, and Veracruz (Beutelspacher and Balcazar, 1994) and therefore in different vegetation types (Gómez, 2009). Finally, some localities like Milpa Alta are characterised by presenting diverse vegetation types like cactus scrub desert, pine forest and fir forest. We must also say that in Mexico there exist diverse approximate equivalences in the nomenclature of vegetation types and that in this case we use as reference the book by Rzedowski (2006).

In Supplementary Table S3, where we show the nutritional value of some forest insects, the grasshoppers present the highest protein values that range between 56.19 and 77.13 g/100 g; nevertheless, *Mischocyttarus* sp. (74.51 g/100 g) and *Proarna* sp. (72.02 g/100 g), are also rich in this parameter. In fat content the insects with the highest quantities are *Phassus triangularis* (77.17 g/100 g) and *R. palmarum* (66.54 g/100 g); *Copestylum haggi* and *Copestylum anna* (8.30 g/100 g) are rich in ash content while *Arsenura armida armida* has 8.23 g/100 g. In the case of raw fibre the most significant results are those of *Callipogon barbatus* (22.71 g/100 g) and *Aplagiognathus* sp. (22.04 g/100 g), and in carbohydrates the larvae of *Hylesia frigida* and the larvae and pupae of *Apis mellifera* have the highest quantities, being respectively 29.53 and 29.46 g/100 g.

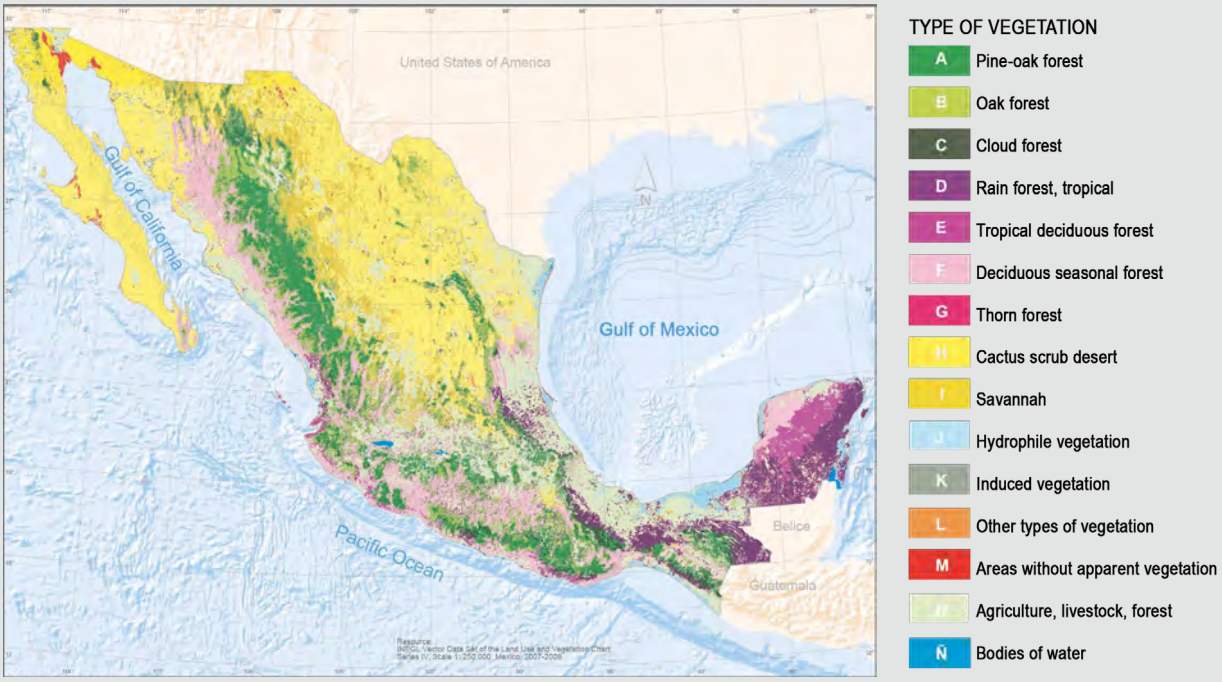

Figure 1. Types of vegetation in Mexico (SEMARNAT, 2010).

Table 1. Distribution of some edible forest insects in Mexico, by type of vegetation (modified from SEMARNAT, 2010).[1]

Type of vegetation	Scientific name
A: pine-oak-forest	*Aplagiognathus spinosus*, *Mallodon dasystomus*, *Mallodon molarius*, *Mallodonopsis mexicanus*, *Derobracus procerus*, *Derobracus* sp., *Callipogon barbatus*, *Leptinotarsa decemlineata*, *Oileus rimator*, *Brachygastra lecheguana*, *Polybia occidentalis bohemani*, *Polybia parvulina*
-A- in Figure 1	Oak forest*: Euschistus taxcoensis*, *Euschistus strenuus (Euschistus zopilotensis)*, *Euschistus* sp., *Edessa mexicana*, *Stenodontes cer. maxillosus*, *Polybia occidentalis nigratella*, *Brachygastra mellific*
-B- in Figure 1	Pine forest: *Hoplophorion monograma*, *Catasticta teutila*, *Eucheira socialis*, *Phassus triangularis*, *Synopsia mexicanaria*, *Arhopalus rusticus*, *Trichoderes pini*, *Apis mellifera*
C: cloud forest (-C- in Figure 1)	*Cerambix* sp., *Atta cephalotes*, *Atta mexicana*, *Atta texana*
D: rain forest, tropical evergreen forest (-D- in Figure 1)	*Mallodon* sp., *Phassus* sp., *Arsenura armida armida*, *Trigona* sp., *Melipona* spp., *Brachygastra azteca*
E: tropical deciduous forest (-E- in Figure 1)	*Hylesia frigida*, *Ascalapha odorata*, Cicadidae, *Rhynchophorus palmarum*, *Passalus punctiger*, *Lestrimelitta limao*, *Parachartegus apicalis*, *Mischocyttarus* sp.
H: cactus scrub desert (-H- in Figure 1)	*Strategus aloeus*, *Metamasius spinolae*, *Scyphophorus acupunctatus*, *Copestylum haggi*, *Copestylum anna*, *Campylostoma* sp., *Liometopum apiculatum*, *Liometopum occidentale* var. *luctuosum*, *Camponotus* sp. *Thasus gigas*, *Proarna sp.*, *Dactylopius coccus*, *Castnia chelone*, *Laniifera cyclades*, *Aegiale hesperiaris*, *Comadia redtenbacheri*
I: savannah (-I- in Figure 1)	*Sphenarium histrio*, *Sphenarium purpurascens*, *Sphenarium magnum*, *Taeniopoda* sp., *Ochrotettix salinus*, *Tropinotus mexicanus*, *Osmilia flavolineata*, *Schistocerca paranensis*, *Schistocerca* sp., *Trimerotropis pallidipennis*, *Melannoplus* sp., *Eushistus egglestoni*

[1] In these types of vegetation we have no records of edible insects: F = decidous seasonal forest, G = thorn forest, J = hydrophile vegetation, K = induced vegetation, L = other types of vegetation, M = areas without apparent vegetation, N = agricultural, livestock, forest, Ñ = bodies of water.

4. Discussion

If we intend to preserve, disseminate and rationally exploit insects as a renewable natural resource, it is necessary to consider their ecological interaction with their respective environments; therefore, taxonomical, biological, ecological (population dynamics), genetic and environmental impact studies must be carried out in order to assess the current situation of their populations and the genetic diversity of the species. It must also be considered that environmentally there is the problem of agrochemicals polluting insects, so that consumers must be aware of their places of origin, as in the case of the grasshoppers. Besides, in Mexico, the current vegetation reflects the great changes it has suffered due to human activities like agriculture, fires, livestock production and deforestation, that cause desertification and the loss of biodiversity in certain regions (SEMARNAT, 2010). For example, logging disrupts insect biology and continuous use of insecticides can cause a decrease in their populations, and in an extreme case, favour their extinction. For example, in the state of Hidalgo, overexploitation of edible insects for socio-economic purposes (commercialisation) is a danger in some areas, as this aspect has gradually reduced the populations of the most sought and commercialised insect species, so that they are now in an endangered status and potentially at risk of extinction. Nevertheless, as has been pointed out by Ramos-Elorduy *et al.* (2006), the main problem is that there is no regulation for their collection, preparation, distribution and commercialisation, as they are equally sold in markets or *tianguis* as well as in five-star restaurants without sanitary control. In this case some researchers believe that creating a wider market for edible insects could provide an economic incentive for the conservation of insect habitats in the forest.

In addition, in Mexico there is almost no information related to the management of edible insects in forest vegetation, so in this case it should be necessary to enhance the forest environments in Mexico and gradually reduce pesticide use and thus reduce pollution. The majority of the edible insects reported in the present study were harvested in small quantities from the forests, in most cases, minimal management of forest vegetation has been practiced in association with the exploitation of forest insects, and actual domestication of insects thus far has been limited to only a few species such as silkworms and bees (Johnson, 2012), even though they represent a significant amount of food for people living near forest vegetation.

Consequently, for the rational exploitation of insects it is convenient to become acquainted with edible forest insect biology, their cultivation and/or breeding, as well as practicing and exploring opportunities for the domestication of forest insects using host plants for feeding them (Balinga *et al.*, 2004; Illgner and Nel, 2000). It has even been pointed out that raising edible insects does not require complex infrastructure, they feed by themselves, plant debris or animal organic matters can be used as manure and their maintenance is easy, they require little space and cause little pollution (Ramos-Elorduy *et al.*, 1988b, 2002b). Therefore, insect breeding offers opportunities that are compatible with forest management. It is important to quantify the impact that insect overexploitation could have on the ecosystems and assess the management of vegetation forest experiences and the insect harvesting practices with the aim of maintaining and maximising the populations. It is also important to gain knowledge about the ecological status of the insect species involved; additionally, the traditional knowledge of the forest inhabitants that still include insects in their diets must be rescued.

Notwithstanding these problems, in Mexico the capture, processing, transportation and commercialisation of edible insects in general, are income-generating activities that potentially improve the life quality of the local inhabitants. In short, there exists an established market and the significant potential of increasing the commercialisation of edible insects. This involves the promotion and adoption of modern standards of alimentary technology so that they can be sold in several innocuous preparations. Some researchers even consider that creating a bigger insect market could provide an economic incentive for the preservation of their habitat, that is, the commercial potential of forest insects must be determined.

Equally important is highlighting once again the advantages that edible insects possess as an alternative to solve the world's alimentary problem (Van Huis *et al.*, 2013) and their management in laboratory conditions may help reduce the environmental impacts that have their origin on stockbreeding. In addition, the nutritional value of various edible species from Mexico has been reported in terms of their contents of protein (essential and non-essential amino acids), fats (saturated and unsaturated fatty acids), vitamins (A, C, riboflavin, niacin) and minerals (Na, K, Ca, P) (Pino and Ganguly, 2016; Ramos-Elorduy and Pino, 1990, 2001a,b; Ramos-Elorduy *et al.*, 1992, 1997, 1998a,b, 2002a,b, 2012).

Consequently, in depth studies will help revitalise the traditional knowledge of several cultural groups, favouring a sense of connection with nature as well as the development of recommendations and strategies for the promotion of forest insects, enabling a rational and sustainable management on a wider scale (Aldasoro, 2000; Durst and Shono, 2012).

Finally, edible forest insects offer a variety of research, conservation and commercialisation opportunities, like contributing to biodiversity maintenance (DeFoliart, 1997); it has been demonstrated that insects contribute significantly to the diets, as in the case of the Yukpa Indians of Colombia (Ruddle, 1973). Even the Food and Agriculture

Organisation (FAO) has pointed out that the exploitation of forests and the associated insects is a very important factor in the fight against hunger, and therefore must be integrated into food security policies, since they contribute to the livelihood of more than a billion people, including most of the world's disadvantaged groups. As an example, insects are the main source of easily accessible protein and honey to the forest dwelling people (FAO, 2013).

5. Conclusions

A wide variety of different insect species are part of the diet of many cultures in Mexico. The use of some edible forest insects by diverse indigenous and *mestizo* groups of rural areas represents rich sources of protein for the improvement of human diet, especially for individuals suffering poor nutrition due to protein deficit; gram per gram, insects often contain more proteins and minerals than meat (Johnson, 2012). These insects, in a smaller scale, are commercialised in the times of highest abundance; their collection is characterised by low production and thus has a minimal impact upon the forest vegetation. But the demand of this product has grown and it has generated new commercialisation opportunities as well as local jobs, so it is important to promote and preserve anthropo-entomophagic activity, aiming at the rescue, conservation, application and promotion of the traditional knowledge so that the management of the insect and plant resources may be exploited viably. For example, we recommend the design of new breeding methods, a 'farm system' or 'mini-husbandry', so as to ensure continuous production and enhance the lifestyle of the local communities.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/JIFF2020.0099.

Table S1. Taxonomic list of some edible insects registered in the forest vegetation of Mexico.

Table S2. Taxonomic analysis of host plants.

Table S3. Nutritive value of some forest insects of Mexico (dry basis g/100 g).

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Conflict of interest

The authors declare no conflict of interest.

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পারিবারিক সম্পর্কের আলোকে 'চোখের বালি'

অসীম কুমার মুখার্জী

ভূমিকা : শিক্ষিত ও সম্ভ্রান্ত মধ্যবিত্তের নাগরিক জীবনচর্যাই রবীন্দ্র উপন্যাসের বিষয় ভিত্তি। সামন্ত ভূস্বামী বা তাদের পিছনে ভোগবিলসিত রাজকীয় জীবন ধারার উৎস সন্ধানে নিযুক্ত ছিলেন বঙ্কিম। সমকালীন জীবন প্রসঙ্গ তাঁর উপন্যাস অবতরণ করেনি। রবীন্দ্র-উপন্যাস কালের হাত ধরেই অগ্রসর হয়েছে। মধ্যবিত্তের বুদ্ধিক্ষেত্র চেতনও বাদ উপন্যাসে স্পষ্ট হয়নি। বরং পেশাবদ্ধ বিশেষ লক্ষণ ছাড়াই মধ্যবিত্তের মানসজীবন ব্যাপ্তির একটি অনিয়ন্ত্রিত ভগ্নাংশই রবীন্দ্র উপন্যাসের প্রধান উপজীব্য। ব্যক্তিই এই কাহিনীর একমাত্র নিয়ন্তা। ব্যক্তিধর্মের বিচিত্রমুখী দিকে দিকে ধরেই রবীন্দ্রনাথ উপন্যাস রাজ্যে অগ্রসর হতে চেয়েছেন। জীবনচিত্রে যেটুকু জটিলতা এসেছে, তার নিয়ামক ব্যক্তিধর্মেরই পারস্পরিক সংঘাত। কখন আত্মতারই বিচিত্র টানে অনিবার্য হয়ে উঠেছে সমস্যা।

ঘটনা ভিত্তির পর বাংলা উপন্যাসকে চরিত্রভিত্তি দান করাই রবীন্দ্রনাথের বিশিষ্ট অবদান। বঙ্কিম গল্প সংগঠনে মনস্তত্ত্ব নিয়োগ করেছিলেন, আজ তারই ফলে আদর্শ প্লটের মডেল পাওয়া গিয়েছে তাঁরই কাছ থেকে। এই প্লট ভিত্তি রচনাই ছিল উপন্যাসের শক্তিবিন্যাসের প্রথম ধাপ। চরিত্র ভিত্তি ঠিক এর পরের প্রত্যাশা। রবীন্দ্রনাথ মনোবিশ্লেষণ পদ্ধতি অবলম্বন করে ব্যক্তির অন্তর রাজ্যকে উন্মোচিত করলেন। উপন্যাসের বিবৃতি ও কথনের প্রধান কেন্দ্রবিন্দু হয়ে উঠল চরিত্র অর্থাৎ ব্যক্তি। সুতরাং ব্যক্তির অধিবাসন ঘটানো কুশলতারই প্রতিশ্রুতি মূল্য রবীন্দ্র উপন্যাসে এরই নানা গতি প্রকৃতি চোখে পড়বে। ব্যক্তির আত্মনিয়ন্ত্রণ অধিকারের অনেকগুলি ক্ষেত্র রবীন্দ্র উপন্যাসে উপস্থিত। ধর্ম, সংস্কৃতি, রাজনীতি, প্রেম, পরিবার-সংগঠন ইত্যাদিকে ঘিরে ব্যক্তির বিচিত্র ইচ্ছা ও ব্যক্তিবিকাশের ক্ষেত্রগুলি রবীন্দ্র রচনায় প্রদর্শিত। 'চোখের বালি'র পারিবারিক কাঠামো ও সম্পর্কের বন্ধন তারই প্রথম নিদর্শন।

মাত্রাপুর : রাজলক্ষ্মী ও মহেন্দ্র

'চোখের বালি' পরিবারিক উপন্যাস : তার সমস্ত ঘটনাবলি পারিবারিক গন্ডির মধ্যে সীমাবদ্ধ। পরিবারের চতুঃসীমার বাইরে যে বৃহত্তর সমাজ 'চোখের বালি' উপন্যাসে আপাতদৃষ্টিতে তার ছায়াপাত লক্ষ্য করা যায় না একটিমাত্র পরিবারকে কেন্দ্র করে কাহিনীর সূত্রপাত — অগ্রগতি ও ক্রম পরিণতি। এই পরিবারের সদস্য সংখ্যা মাত্র চার জন রাজলক্ষ্মী-মহেন্দ্র-আশা-ও অন্নপূর্ণা। এই চতুরবর্গের মাঝেই কাহিনী বিন্যস্ত হয়েছে। মাতা রাজলক্ষ্মী একমাত্র পুত্র মহেন্দ্র পিতৃহীন মায়ের স্নেহ ছায়ায় বেড়ে ওঠা। আদর আবদার যাই হোক সবই মাকে নিয়ে। বিধবা বাল্য সখী হরিমতির কন্যা বিনোদিনীর সঙ্গে রাজলক্ষ্মী পুত্রের বিবাহ দিতে চাইলে মহেন্দ্র তাতে অসম্মত হয়। কিন্তু এসে যদি মাকে ছাড়িয়ে উঠে এই ভয়ে মহেন্দ্র অনেকদিন বিবাহ করলে না। "মা-সম্বন্ধে তাহার ব্যবহার সকল লোকের মত ছিল না। বয়স প্রায় বাইশ হইল। এম.এ পাশ করিয়া ওকালতি পড়িতে আরম্ভ করিয়াছে, তবু মাকে লইয়া তাহার প্রতিদিন মান-অভিমান, আদর-আবদারের অন্ত ছিল না। কাস্যাক্ত শাবকের মত মাতৃগর্ভ হইতে পৃথিবী হইয়াও মাতার বহির্গাত্রের ঝিল্লিটির মধ্যে আবৃত থাকায় তাহার অভ্যাস হইয়া গিয়াছিল। মার সহায়তা ব্যতীত কোন

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Green microalgae derived organic nanodots used as food preservative

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ABSTRACT

Spirulina platensis derived organic nanodots (ND) may be used as an alternative of chemical food preservative that can increase shelf life of food and beverages. Microalgal nanodots were isolated by the supercritical carbon dioxide extraction (SCE) method. Then SCE extract was subjected to thin-layer chromatography (TLC) and fractions were screened by a well diffusion method to identify the active compound with strong antibacterial ability. A characteristic of ND is the emitting of red colour upon the absorbance of ultra-violet (UV) light. The isolated ND is active against both Gram-positive and Gram-negative bacteria that significantly avoid their auto-aggregation. The nanodots were characterized by using TEM, UV-VIS, FTIR and NMR spectroscopy. FTIR and NMR analysis revealed the presence of hydrogen-bonded hydroxyl and amide groups. In addition, ND is applied in litchi juice as a preservative to extend the shelf life. In general, our results revealed that the sensory quality i.e. colour, aroma and overall appearance were significantly affected by duration of storage. It proves that *Spirulina platensis* derived organic nanodots act as a strong food and beverage preservative.

1. Introduction

Few of traditional chemical preservatives act as chemical contaminant of food, during adverse effect in human health. Because of our society can find out an alternative way for bypass of chemical pollutants of food. As of now, food preservatives are important to reduce food spoilage caused by microorganisms preventing loss of its quality and nutritive value. Recently, it has created a major global concern due to the excessive and indiscriminate use of antimicrobial compounds; resulting pathogens have developed resistance via genetic mutation or gene acquisition. Therefore, new types of safe antimicrobials urgently require combating this trend. Nevertheless, nano particles have various exceptional properties, viz. large unique nano size, surface area/volume ratio and antimicrobial properties. Efforts are given for the biogenic and rational design of metal or peptide based nanoparticles for their use as antimicrobial agents [1–3]. In this time, novel nanodots (ND) have increased great interest in biological fields owing to the unique size-tunable light absorption and emission properties. The light emission ability of ND can be adjusted through their size and shape with extraordinary biochemical properties. The ND is also highly photo stable than synthetic organic fluorescent dyes. It has a profound wide range of applications in biological sciences with remarkable healthcare systems due to their unique optical and electronic properties [4]. During the past century, exhaustive research has accounted for countless benefits on nanomaterials. Nanomaterials are used in several sectors, among them the food industry is one of the prime industries. Even though, the need of nanotechnology in the food industry has prominently increased day by day. Nanotechnology assures various benefits like safety, security, quality and shelf life of the food products in food processing industries. The application of nanomaterials in food technology is based on the characteristics of the nanoparticles. On the other hand nano-food processing is accomplished in three main aspects i.e. production (conversion of edible food materials from raw food), processing (enhance of taste, shelf life and safeguard from contaminants) and maintenance of proper nutritional standards. It also plays an efficient role in food preservation and packaging i.e., use in sweets, chewing gums and candies preparation. As of now, the production of nanoparticles has been reported by many photoautotrophic microorganisms such as cyanobacteria, eukaryotic algae, and fungi [5].

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Several scientific evidences proved that the biogenic nanoparticles production via micro algae is considered to be a harmless and eco-friendly green chemistry technique with the potential variety of compositions and physicochemical properties [6]. It is widely documented that green seaweed is used in agriculture, pharmaceutical, biomedical and nutraceutical industries for its presence of high amount of vitamins and minerals. Among several genera of microalgae *Spirulina platensis,* blue green microalgae of cyanobacteria family has grown in temperate waters around the world. A few cross-sectional investigations support that blue green microalgae has been used as a source of food supplement for its high protein content and nutritional value from ancient times [7]. The microalgae produce novel and potentially useful bioactive compounds. Recently the presence of the bioactive compounds has concerned significant interest resulting in the synthesis of new pharmaceutical products, food products, biomedical applications and renewable bioenergy [8]. There are various conventional extraction techniques using hexane, ethanol and water for the collection of bioactive molecules. But they are very problematic due to instability as well as environmental and health hazards. At this time to overwhelm this problem the researchers developed a new approach i.e. supercritical fluid (SCF) extraction technology for avoiding toxic organic solvents in green technology [9]. SCF possesses physical properties intermediate between CO_2 gas and a liquid at a temperature and pressure above its critical point. Besides that, addition of a small amount of co-solvents can cause swelling of algal cells, facilitating the rapid mass transfer of analytes from the matrix [10].

In this research study, we have developed a new way to isolate the organic nanodots from edible freshwater blue green microalgae, *Spirulina platensis.* This is the first report for the production of organic ND from *S. platensis* and their application as food preservative.

2. Materials and methods

2.1. Collection of sample

During this research study *Spirulina platensis* var. lonor was collected from Antenna Green Trust, Madurai, Tamil Nadu, and India. Samples were stored under dry and dark conditions in the sealed container for further analysis.

2.2. Cultivation of bacteria

In laboratory cultures conditions both Gram-positive and Gram-negative food poisoning bacteria i.e. *E. coli, B. subtilis, S. aureus* and *V. cholerae* were selected for anti-bacterial experiment [11]. The appreciable amounts of autoclaved Muller Hinton Agar (Hi-Media) media was dispensed into sterile plates and allowed to solidify under aseptic conditions. Both types of bacterial strains were spreaded on agar containing sterilized plates. Then the plates were incubated at 37 °C for 24 h. After that the developed bacterial colonies were transferred to nutrient broth medium (Hi-Media) and incubated in a shaking incubator at 37 °C for 24 h.

2.3. Bioactive molecule extracted by supercritical carbon dioxide extraction method

The supercritical carbon dioxide extraction of *Spirulina* microalgae was done by means of a Speed_SFE System of Applied Separations, USA extraction unit. The system comprises a pump fitted with a refrigerated cooling bath to chill the pump head at −4 °C. In case of long run, 50 g (50 g) of freeze dried *Spirulina* powder was weighed accurately in a weight balance. Then it was packed into a 100 ml stainless steel vessel. The glass wool was to be put at both ends of the extraction vessel to avoid the leakage from the vessel. After that the compressed CO_2 was passed from the bottom most of the vessel at a flow rate of 300 ml/min using micrometering valves. A co-solvent pump of 100% ethanol was added in the extraction vessel at a flow rate of 1 ml/min. The extraction pressure and temperature were set at 200 bar and 35 °C respectively. According to the experimental set up the total time was fixed as 120 min according to static (60 min) and dynamic extraction time (60 min). After dynamic extraction time microalgae extract was collected in a SFE glass vial. During all extractions conditions the outlet valve temperature of SFE system was regulated greater than the oven temperature to avoid the break of glass vials owing to the increase of ethanol vapours and compressed CO_2 [12].

2.4. Isolation and purification of bio-active compound from microalgae extract

The extract was subjected to thin layer chromatography (TLC) plate to fractionate and isolate the active compound from microalgae that has shown antibacterial activity. A running solvent (acetic acid: butanol: water = 1.5:6:2.5) was used for the separation of an active compound from microalgae. An aliquot of SFE microalgae extract was dotted on the TLC plate using a micro needle. The spots were allowed for dryness. Then the plate was positioned in a TLC chamber containing the running solvent. After that when the solvent was touched approximately 1 cm from the top, TLC plate was removed from the solvent and dried at 45 °C. Pictures of the developing red colour spots in TLC plate were taken. These colour spots were scraped and dissolved in ethyl acetate to isolate the compound from the *Spirulina* biomass extract [13].

2.5. Isolated compounds characterization

2.5.1. Functional group detection by fourier transforms infrared spectrometer (FT-IR) study

According to the method of Jagmohan, 2005 the FT-IR studies have been performed. The potassium bromide (KBr) pellet was added to the sample [14]. Then the sample was placed in a transparent disc of thickness 1 mm and diameter 13 mm and subjected to about 5 × 106 Pa pressure in an evacuated die. IR spectra region of 4000–500 cm^{-1} were recorded on a FT-IR Nexus TM 870 spectrophotometer (PerkinElmer) [15].

2.5.2. Structural analysis by transmission electron microscope (TEM)

The microalgal sample with concentration of 1 mg/ml was drop casted on a Cu-grid. After drying at room temperature, the samples were directly viewed under TEM following the guidelines as described earlier by Mahata et al., 2017 [16]. The HR-TEM (JEOL JEM 2100 instrument) was used to analyze the samples and capture the images.

2.5.3. Structural analysis through nuclear magnetic resonance (NMR) spectroscopy

^1H NMR can be used to study the presence of hydroperoxy groups as well as of hydrogen, carbonyl and carbon atoms associated in conjugated-dienoic systems. Data was recorded on a DPX200 Bruker (200 MHz for ^1H and 500 MHz for ^{13}C) in D_2O with tetramethylsilane (TMS) as an internal standard. Every signal was compared within ±0.1 ppm to standard solution [13].

2.5.4. Antimicrobial activity assessment of bio-active nanodots

The Luria broth (LB) agar plate was prepared with 30 μl inoculums and different doses of active fractions from 1 to 250 μg/ml. Then the agar plates were incubated for 24 h at 37 °C for bacterial growth. Negative controls were prepared adding 30 μl of 50% ethanol in 20 ml LB medium as the solvent used to prepare the ND. The minimum inhibitory concentration (MIC) values were recorded where no visible growth was observed [16]. The plates without compound were treated as positive control for each bacterial inoculums.

2.5.5. Determination of auto-aggregation ability of selected bacteria

Auto-aggregation experiment of bacteria was conducted by use of nutrient broth medium. Selected strains of bacteria, *Vibrio cholerae* were

cultured in nutrient broth in 30 °C at 120 rpm in a shaker incubator. The proper growth of bacteria was checked by measurement of the optical density at 600 nm (OD_{600}) through using a spectrophotometer (VARIAN, INC. Carry^R 50 Bio). Without centrifugation an aliquot of cell suspensions of 1.5 ml were taken for measuring the OD at 600 nm, other 1.5 ml aliquots were separated and centrifuged at 650×*g* for 5 min. After that supernatant was collected and measured the OD at 600 nm. The OD at 600 nm of the samples was recorded three times. The bacterial aggregation index was then calculated with average value as [17]:

$$\text{Aggregation index} = OD_{(Total)} - OD_{(Supernatant)} / OD_{(Total)}$$

2.6. Shelf life estimation of litchi beverage preserved with purified compound

From the local market ripe litchi were collected. During proper grinding and filtration by muslin cloth litchi juice was extracted. Before heating on low flame, 2% sugar was added with prepared juice. Then ND was added as preservative in litchi juice. The treated juice was filled in a glass bottle under sterilized conditions. The glass bottles were shielded immediately using a hand shieling machine. Finally, a total two sets of studies were conducted by varying storage temperature viz. refrigerated (4 ± 2 °C) and ambient storage (room temperature) at specific time interval (0, 7, 15, 30, 45, 60 days) [6].

2.6.1. Physico-chemical analysis of litchi beverage

The litchi juice samples were taken from bottles at specific time intervals and were analysed for total bacterial counts, total fungal count, total soluble/suspended solid, pH, acidity. The pH of the litchi juice was determined with the help of a digital pH meter (Systronics, Model 361). According to the method of Sivakumar and Korsten, titratable acidity was estimated [18]. At first 5 ml of litchi juice was taken and diluted to 50 ml using milli Q water. 100 ml of 0.1% phenolphthalein was then added to 10 ml of diluted juice. 0.1 N NaOH was used to titrate juice containing solution. After a certain period the pink coloration appeared. The volume of used 0.1 N NaOH in titration was noted as the titre value. The titratable acidity of juice was calculated as citric acid percentage. Total soluble solids (TSS) was determined using hand held refractometer (ERMA, Japan; 0–32) at room temperature [19].

2.6.2. Estimation of total bacterial and fungal count of storage litchi beverage

Total plate counts (TPC) were estimated on nutrient agar media (Hi media). The serial dilution of the sample was made sequentially and plated on LB agar plate. After incubation at 37 °C for 24 h colony forming units (CFU) were counted. Fungal counts were estimated on potato dextrose agar (Hi media) after serial dilution of sample. After incubation at 28 °C for 72 h CFU were counted [20].

2.6.3. Sensory evaluation of storage litchi beverage

The litchi juice was selected by sensory evaluation on 9 point Hedonic Scale for different sensory attributes like colour, flavour, appearance and overall acceptability by a panel of 10 non-smoker judges having prior experience of sensory evaluation of fruits and vegetable products. A scale from 1 to 9, where 1 represented extremely disliked and 9 represent extremely liked [21].

3. Results and discussion

The following results of the assessment of organic nanodot as food preservative from *Spirulina platensis* are presented and discussed. This assessment is built upon several sections to analyze and inform.

3.1. Isolation and purification of bio-active compound

The supercritical fluid extract of *Spirulina platensis* biomass (Fig. 1a and b) was fractionated by TLC analysis (Fig. 1c). The active fractionate was then separated from the silica plate and dissolved in ethyl acetate. The solution was further centrifuged at 8000 rpm to separate the active purified compound (Fig. 1d) and lyophilized for further analysis. TEM analysis revealed the average size of the ND is 40–45 nm.

3.2. Spectral characterization of the purified bio-active compound

Ultra violet-visible (UV-VIS) spectroscopy usually provides the characterization of nanodot molecules. When white light is passed through a coloured substance, a distinctive portion of the mixed wavelengths is absorbed. The residual light then will assume the complementary colour to the wavelength (s) absorbed. The ultra-violet spectral absorbance of purified compound was found to be λ_{max} at 400 nm which is corresponding to the presence of olefinic chromophore (conjugated) (Fig. 2a). Bio-material planes can be observed as a variety of functional groups that are responsible for association of metal ions, with hydroxide (–OH), amide (–NH2), thiols (–SH), carboxylate (–COO–), and phosphate (PO_4^{3-}) [22]. The IR spectrum at 3307 cm^{-1} signifies the ranging vibration of hydroxyl groups. The C–H stretching of vibration indicated at 2986 cm^{-1}. The spectra at 1751 cm^{-1} and 1638 cm^{-1} were recognized to the stretching of C═O vibrations (Fig. 2b). The compound has a signal for C–H aliphatic protons that involved with O- atom at 3.31 ppm. The aliphatic protons signals were further shown at 2.17–0.85 ppm region. The ^{1}H NMR spectrum also indicated the absence of aromatic proton signals (Fig. 2c). The ^{13}C NMR was consistent with the skeletal structure (Fig. 2d).

3.3. Assay of antimicrobial activity of purified compound

Antimicrobial activity of the nanodot was tested against four bacterial pathogens such as *E. coli*, *B. subtilis*, *S. aureus* and *V. cholerae* (Fig. S1) following agar well diffusion assay. The result revealed that 250 mg/l concentration of nanodot exhibits a small clear zone around the well. Minimum inhibitory concentration (MIC) was determined following serial dilution assay and was observed 1 mg/ml for *E. coli*, *V. Cholerae* and 500 mg/l for *S. aureus and B. Subtilis* [13].

3.4. Assessments of auto-aggregation ability of compound

Communication among same bacterial species is very much necessary for initial colonization and subsequent their biofilm formation [23]. Therefore, auto-aggregation is the initial step of planktonic cells to develop biofilm over there. Control of auto-aggregation is the easiest way and first step to control the biofilm inhibition. In auto-aggregation, bacteria of the same type form clumps to multicellular structures that ultimately settle down at the bottom of culture tubes. The auto-aggregation is generally done by self-interaction of cell surface molecules, such as proteins and exopolysaccharides [24]. The auto-aggregation ability of targeted nanodots was assessed at different time periods throughout the growth phase. Bacterial auto-aggregation ability was determined by a specific aggregation index. For auto-aggregation study, a food borne pathogen, *V. cholerae* was selected. Here, the obtained result revealed that auto-aggregation was increased in the control group (without ND) in comparison to the treated sample (ND at 250 mg/l) (Fig. 3). Lower auto-aggregation means to inhibit the biofilm formation.

3.5. Changes in quality of litchi beverage

The different physico-chemical and biological quality parameters of litchi beverage using preservative (ND) under refrigerated and ambient temperature were studied. The good quality of food product was first

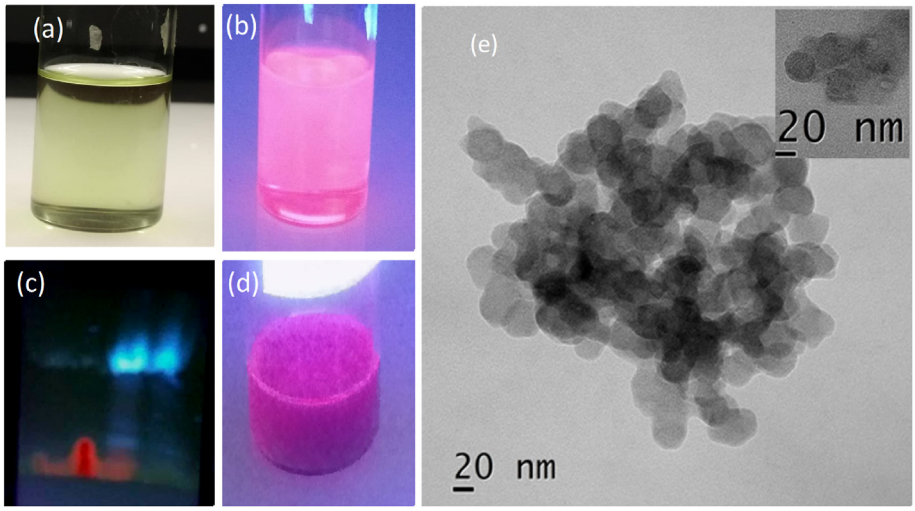

Fig. 1. Separation of active compound. Supercritical fluid extract of *Spirulina platensis* in visible light (a), under UV light (b), The selected area of TLC plate (c), The selected area was collected and concentrated for structural characterization (d), TEM analysis of ND (e).

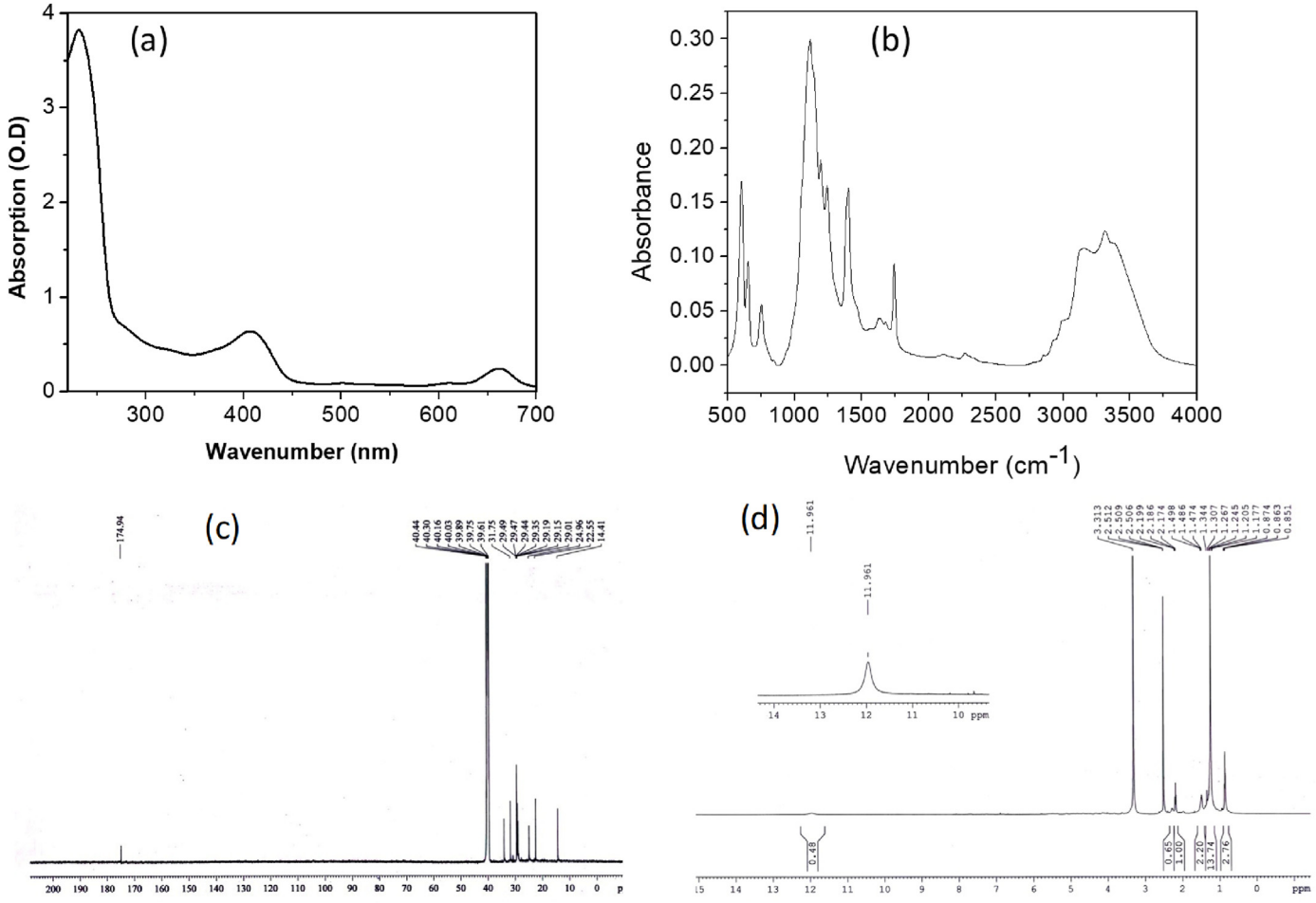

Fig. 2. Spectral characterization of the synthesized NDs. UV–Visible spectrum (a), FT-IR spectrum (b); ^{1}H NMR spectrum (c) and ^{13}C NMR spectrum of purified ND (d).

determined by pH. The Fig. S2 showed the changes of pH values in the litchi juice samples during storage. It was observed that with the increase of storage period and temperature, pH decreased whereas the acidity and TSS were increased. Data revealed that at refrigerated storage on day 30th, pH decreased for ND sample. TSS of the juice was observed to be higher in ambient storage, being 16.8° Brix as compared to 11.8° Brix at refrigerated storage. On day 30th acidity of juice increased at refrigerated storage.

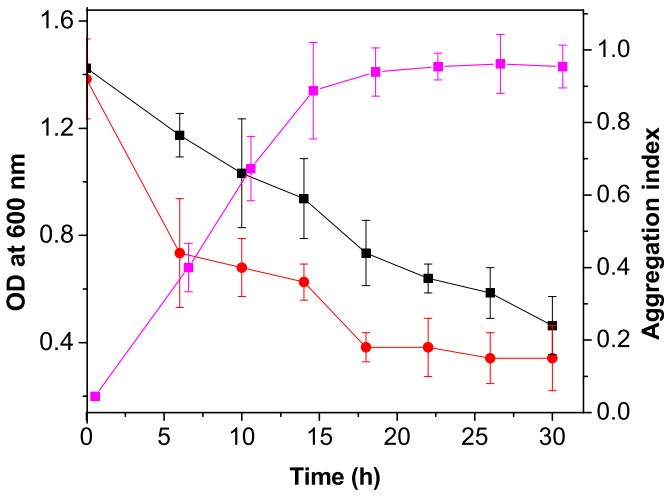

Fig. 3. Determination of auto-aggregation index in the presence and absence of compound NDs. Growth curve of bacteria *V. cholerae* represented the lines with pink colour, auto-aggregation index without NDs (black line) and auto-aggregation index with NDs (red line). Data are the mean of triplicates ± S.E. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.5.1. *Microbial count*

At ambient storage more microbial growth was observed as compared to storage at refrigerated condition. It was found that total bacterial and fungal counts are less in ND treated sample than untreated ones (Table S1 and Table S2).

3.5.2. *Shelf life analysis of litchi beverage*

The suitability of food products during refrigerated storage is related with the variations in their sensory properties. As assessed by the sensory panel, the scores for colour, appearance, taste, odor and overall acceptability of the litchi beverage decreased significantly during the storage period. The results presented that the beverage fortified with organic ND significantly have better sensory scores. The storage time of the treated and untreated juice was judged on the basis of sensory quality. The sensory score of 6 and above on 9 point Hedonic scale rating was used as a threshold for estimating of shelf life for beverage preservation [25]. The analysis of the data showed that storage condition and treatment had a significant effect on overall acceptability of litchi juice. The sensory quality i.e. colour, aroma, and overall appearance were significantly affected by duration of storage. The overall quality changes of the juice with various storage times at refrigerated and ambient conditions were presented in Fig. S3.

4. Practical application of this research study

For instance, nanotechnology has been used for food quality improvement, shelf-life extension, cost reduction, and nutrition enhancement. Exploration of naturally occurring nanostructures in food is one of the hottest topics in the scientific community.

Although orally administered nonmaterial get absorbed and move across the gastrointestinal tract and distributed in the body parts like kidney, liver, spleen, brain. But *Spirulina platensis* derived organic nanodot is easily digested in our gastrointestinal tract during variation of pH and ultimately undigested molecules are released through defecation (Fig. 4). Generally nanomaterials having size around 5 nm and present in blood circulation undergo renal clearance. Those having 10–20 nm are separated by liver; 200 nm sized NPs get picked up by Kupffer's cells and sinusoidal spleen. Higher concentrations of nanomaterials were excreted via kidney, hepatobiliary pathway. Prior research proved that nano products of ZnO and Ag significantly increase the shelf-life of orange juice and beer through preventing contamination of food and beverages because of their antimicrobial nature against *E. coli, Pseudomonas aeruginosa* and *Aspergillus niger* [26].

Since gut bacteria play a critical role in maintaining human health, nanoparticles in food could result in adverse health effects through changes in the gut microbiota. To the best of our knowledge, there are no reports on the impact of ND on gut bacteria. In contrast, the antibacterial abilities of

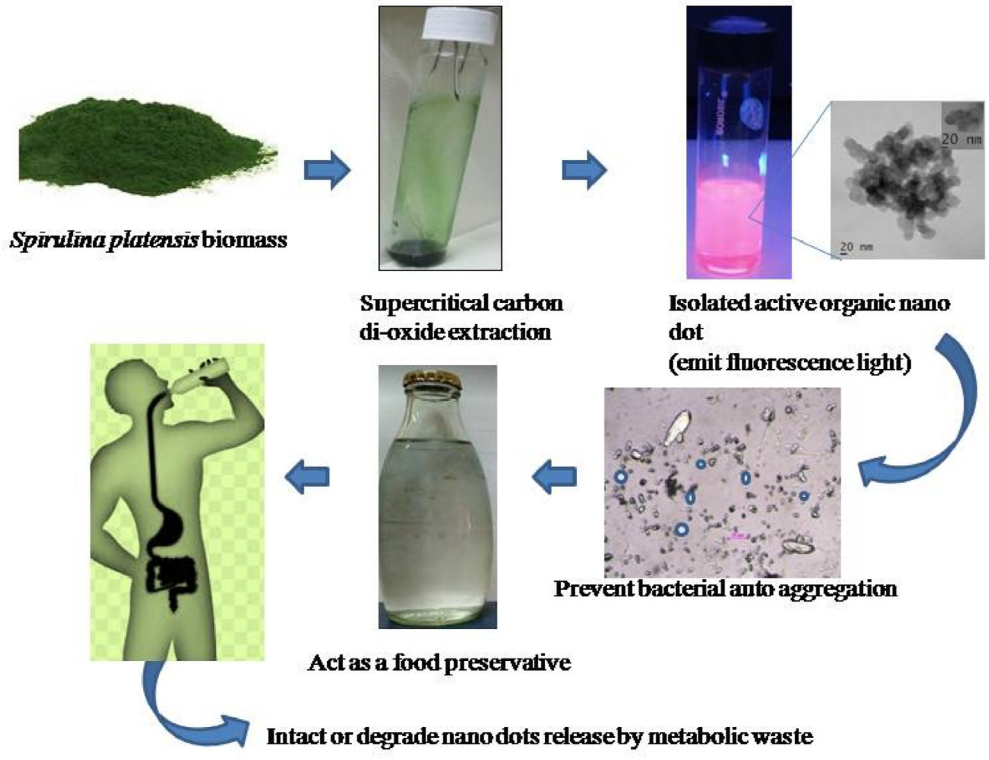

Fig. 4. Organic nanodot synthesized from *Spirulina platensis* used as a preservative.

ND have been demonstrated. The antimicrobial ability was affected by surface groups, charge, shape, and size of ND [27]. Our knowledge about the bio-effects of food borne organic ND is still in its infancy. *Spirulina platensis* derived ND being an organic molecule has a large surface area but very small in size (about 40–45 nm), that can easily penetrate into microbial cell [28]. Thereafter this is important to meet the criteria of food preservative. In view of all these considerations, longer experimental studies are needed to test the durability of the ND with proper efficacies for their reuse as preservative. However, compared to commercial preservatives, ND is considered a low-cost technological approach which can also become cost-effective and beneficial from the commercial perspectives. Food preservation has emerged as an efficient option for food storage but many challenges and constraints with regard to its practical applications on a large commercial scale still prevail. The following points are needed to be addressed to get the adequate benefit of the preservation methods for practical applications in food and beverages storage.

1. Food borne disease and toxicity of chemical preservatives in food are serious threats to human civilization. The feasibility of food preservation by cost-effective and efficient technique should be explored.
2. The mechanism of antimicrobial activities of *Spirulina platensis* derived nanodots are still not fully clear which are required to be understood with more transparency in the perspective of food preservation.
3. The culture of *Spirulina* is an economically viable approach for food preparation and thereby, their use under strict monitoring can be recommended for agricultural applications parallel with bio-remediation of wastewater [29].
4. Bio-energy (hydrogen) production from *Spirulina* alongside undertaking food preparation can become a unique approach that will not only generate food but also lead to the generation of eco-friendly fuels. This technology must be further explored with the aim at achieving possible commercialization [30].
5. The use of algal derived nano based bio-composites may become an effective tool for the adsorption of toxic metal [31].

5. Future prospect

In the face of a significantly growing human population and with the scarcity of healthy food, the world has been facing the challenge of safeguarding human health. Future research studies about the development of *Spirulina* derived nanodots should be focused on food preservation, which demonstrate much better efficacy than traditional ones. In addition, different scientific facts generated from the present research study have touched upon and established the forthcoming multidimensional application of *Spirulina* derived organic nanodots for preservation of food and beverages and thereby further investigations are required to find out the proper mechanism in food preservation, mainly for the under mentioned reasons:

1. The existing output of this research holds greater promise in predicting the antimicrobial potential of *Spirulina* derived nanodots and their practical applicability in food and beverage preservation.
2. Based on earlier information alongside the current research findings, it can be concluded that the nanodots of *Spirulina* can increase shelf life of food and beverages during potential antibacterial as well as anti-fungal activities.
3. Nonetheless, there is a need to undertake more in depth studies with proper chemical analyses that are proposed to constitute the foundation of future research ventures.
4. Organic nanodots will alternatively be used as chemotherapeutics when human pathogens are resistant to antibiotics.

Even though, the traditional chemical food preservatives are also available in the market to increase the shelf life of beverages. Sodium benzoate (SB) can inhibit the growth of spoilage microbes in beverages i.e. *Saccharomyces cerevisiae*, Lactobacillus plantarum. It has been also reported that SB is more effective against yeasts during the 1st day of storage (2–2.5 log CFU reduction) and the microbial growth reduction rates were highest up to 15th day [32]. But those have different side effects including allergy, hypersensitivity, and neurological disorder [33]. Parabens, benzoic acid (BA) and sorbic acid (SA) are commonly used chemical preservatives that are used in beverages. It is reported that among them parabens are esters of the p-hydroxybenzoic acid that have endocrine and reproductive toxicity [34]. Due to such side effects the chemical preservatives are not too recommendable in so many cases whereas organic nanodots have achieved popularity for its no side effects. It was found that total bacterial and fungal counts are less in ND treated sample than untreated sample on 30 days of storage time during refrigerated condition and 15 days during ambient storage. Given such a proposition, it can be concluded that *Spirulina* derived organic nanodots, may also be recommended for the ideal food and beverage preservative. We trust that this research article will pave the way for developing better economically viable as well as significantly acceptable techniques with the focus on food preservation.

6. Conclusion

The present study has established the roles of organic nanodots from microalgae; *Spirulina platensis* holds great promise having numerous potential applications in food industries. This research has opened up new vistas by identifying and establishing the potential efficiency *Spirulina platensis* derived organic nanodot as the food preservative that reaches in several bioactive phytochemicals. Thus this research work is essential to endure the ND research more precisely to progress their surface functionalization and conjugation with bio-molecules. It can also be summarised under four major facts: 1) SFE extract of *Spirulina platensis* was found to produce organic nanodot; 2) nanodot molecule significantly inhibit both Gram positive (*B. subtilis* and *S. aureus*) and Gram negative bacteria (*E. coli*, and *V. cholerae*) as well as fungal contaminants; 3) the organic nanodot able to prevent bacterial biofilm formation; 4) nanodot showing a significantly better sensory quality and enhance shelf life of litchi beverage.

Declaration of competing interest

Authors have no conflict of interest. There is no financial involvement in this work.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crgsc.2022.100276.

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Ammonium phosphomolybdate [$(NH_4)_3PMo_{12}O_{40}$] an inorganic ion exchanger for environmental application for purification of dye contaminant wastewater

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ABSTRACT

Yellow ammonium phosphomolybdate (YAPM), a waste product of all under graduate (UG) chemistry laboratory classes, has been proved to be an efficient adsorbent for environmental application as a cationic ion exchanger. The water insoluble YAPM selectively removes cationic dye molecules from dye contaminated wastewater. Consequently, dye infested YAPM solid is produced with fascinating color but bears altered band gap energy. Dye contaminated yellow APM is regenerated in air oven from the dye infested YAPM solid powder by heat treatment at ~ 300 °C and reused with full potential. So it becomes a solid phase extractant. Robust yellow APM turns to green APM (GAPM) upon UV light exposure because of the hoarded electrons in the basket like Keggin structure of phosphomolybdate ($PMo_{12}O_{40}^{3}$) moiety, and thereby the dye exchange capability is improved, admirably. Thus, the remediation of dye contaminated wastewater by YAPM has been found to be fruitful by YAPM and more potential dye remediation by GAPM.

1. Introduction

Polyoxometalates (POMs) are extremely large group of anionic clusters with frameworks built from transition metal oxo anions linking shared oxide ions [1]. Most of the elements in the periodic table can be incorporated as cations into the structural framework of these robust compounds. Yellow ammonium phosphomolybdate (YAPM), $[(NH_4)_3PMo_{12}O_{40}]$ is a low cost polyoxometalate compound, and a waste product of the undergraduate (UG) laboratory. We have shown useful application of YAPM vis-à-vis UV exposed YAPM i.e., GAPM for purification of dye contaminant wastewater through ion exchange method. Here inorganic ammonium phosphomolybdate acts as an ion exchanger.

Dye contaminated wastewater effluents comes from different industries and that causes a global problem. Particularly cloth-dying industries discharge a huge amount of waste organic dye molecules into rivers without any scientific treatment. Public news media reported serious water pollution caused by organic dye molecules in different rivers of many countries. China [2], India [3] and Bangladesh [4] suffer from this problem. Thus many rivers flows with different organic chemicals, and as a result flora and fauna are affected. In future, it will be a serious problem for aquatic environment as well as human life. To save the clean environment, proper treatment of wastewater is a challenging task to the environmental scientists. There are so many well-known processes for removal of dye molecules from the water bodies. Photocatalysis [5], adsorption [6] and ion exchange process [7] are widely used methodologies. In general, metal oxides [8], sulphides [9] are used as photocatalyst and ion exchangers [10] for demineralization of water. Photocatalytic reaction for purification of dye contaminant wastewater requires visible or UV light for photo-assisted mineralization reaction to proceed. A proper light source (visible light [11] or UV light [12]) is required depending on the nature of dye and band gap of photocatalyst. But in the dark, an adsorbent becomes appropriate than a photocatalyst for dye removal from wastewater. The traditional adsorbents are activated carbon material [13], oxides [14] and sulfides [10] which are used for purification of water bodies. Recently, researchers have exploited CNT [15], graphene oxide [16], reduced graphene oxide [17], organic gel [18] and inorganic gel [19] materials for dye removal

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by adsorption of dye molecules since these materials often bear large surface area. Generally, forces, operate between the adsorbent and adsorbate dyes may be hydrophobic interaction, [20] hydrogen bonding attraction [21] and electrostatic force [22] in ion exchange processes. The absorbent with ion exchange property is a better option than adsorbent with non-ion exchange capability as in the latter case there happens stronger electrostatic attraction, selective binding minimization of leaching of adsorbate happen from the absorbent.

Junbai Li et al. reported that MnO_2 hollow sphere can separate congo red effectively from dye contaminated wastewater [14]. Purely inorganic material, indium selenide $(NH_4)_4In_{12}Se_{20}$, has cation ion exchange property which can remove different toxic metal ions such as Cs^+, Rb^+, Hg^{2+} and Pb^{2+} *etc.* via electrostatic interaction [23]. So they are used to purify these heavy metal ions from water bodies. An important layered sulfide material $K_{2x}Mn_xSn_{3-x}S_6$ ($x = 0.5$–0.95) which bears highly mobile or exchangeable K^+ ions in their interlayer spaces. So, K^+ is easily exchanged with other toxic metal ions such as Sr^{2+} ion [24], UO_2^{2+} ion [10], Hg^{+2} ion [25], Cs^{+1} ion [26] *etc.* via electrostatic force that helps purification of contaminated water reserves.

In this article, we have reported a unique method for removal of cationic dye molecules using water suspension of low cost and readily available yellow ammonium phosphomolybdate $[(NH_4)_3PMo_{12}O_{40}]$ (YAPM) and also UV exposed green ammonium phosphomolybdate (GAPM) which is proved to be a more efficient inorganic ion exchanger [27,28]. After dye exchange reaction, the dye infested YAPM upon heat treatment at ~300 °C regenerates the parent adsorbent, YAPM with the retention of original chemical stability and recyclability. The electrostatically bound dyes are not at all leached out from the adsorbent, YAPM. The most interesting phenomenon is that the yellow APM is photoreduced to green ammonium phosphomolybdate (GAPM) upon UV (~360 nm) light irradiation. As a noticeable result, GAPM acquires more effective dye removal capacity again through ion exchange reaction step. It is observed that the dye exchange capacities of YAPM and GAPM have higher values than common polystyrene based cation exchange resins. However, the dye removal capacity varies from dye to dye. In the present investigation, different types of dye molecules (such as cationic, anionic and neutral dye) are used for exchange reaction with APM materials. Among them methylene blue (MB), malachite green (MG) and rhodamine B (RhB) have experienced exchange reaction with $[(NH_4)_3PMo_{12}O_{40}]$ material as all these dye molecules are cationic in nature. Ammonium ion (NH_4^+) in APM becomes exchangeable with only the cationic dye molecule. So the yellow ammonium phosphomolybdate $[(NH_4)_3PMo_{12}O_{40}]$ (YAPM) and UV exposed GAPM find novel environmental application as inorganic ion exchanger for cationic dye removal from water bodies. Dye infested APM molecules are readily recovered by heat treatment. Thus the method proclaims a unique 'zero waste' strategy for dye removal from wastewater with 100% APM recovery by heat treatment.

2. Experiment and Methods:

2.1. Materials and instruments

The material and instruments are given in the supporting information SI 1.

2.2. Preparation of yellow APM and green APM

In a typical preparation, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (4.50 g) and $NaH_2PO_4{\cdot}H_2O$ (0.50 g) were added into 200 mL distilled water and warmed on a water bath at ~ 80 °C to obtain a clear solution. Then the warm solution on the water bath was acidified with conc. HNO_3 (3 mL). The solution was stirred by a glass rod for 1–2 min and kept for the next 30 min at the same temperature. The canary yellow material was precipited and washed several times with distilled water until its pH value lowers down to ~ 6.8 (pH of distilled water) and dried in an air oven at 100 °C. Due to the high water solubility of the parent salts, solid and crystalline form of the synthesized material was washed several times with water. So impurity contamination in the synthesized material becomes redundant. The yellow product is ammonium phosphomolybdate, $[(NH_4)_3PMo_{12}O_{40}]$. The yellow APM material was exposed to UV light (~365 nm with spectral distribution of 350–400 nm) of power 15 W for 30 days in Air in UV-light. About 1 gm APM was spread as a thin layer on large size petri dish and placed in the UV-light chamber for long time (30 days). Every day, the material was respreads and exposed to UV light and continued for 30 days to ensure the complete surface reduction of yellow APM.

The whole material (YAPM) changed to deep green APM (GAPM) by solid state photochemical reaction. The green APM i.e., GAPM [27,28] was stored in the dark under inert condition (N_2 or argon gas). Upon storing the GAPM sample in an open place at room temperature condition, the material gradually slowly reverts back to YAPM. The green and yellow materials (YAPM and GAPM) were employed as inorganic exchangers for dye removal from wastewater through ion exchange reaction.

2.3. Kinetic studies of dye exchange reaction

The organic dye molecule bearing positively charged structure was used for ion exchange experiment with YAPM. The yellow matrix electrostatically exchanges its NH_4^+ ion with the incoming cationic dye molecules. We have employed four dyes [methylene blue (MB), malachite green (MG), rhodamine B (RhB) and methyl violet (MV)] for the ion exchange reactions to study the efficacy of APM towards the dyes. The progress of dye exchange reaction was monitored by a UV–Visible spectrophotometer at various time intervals.

For each experiment, a total of 100–200 mg of YAPM was weighted in a 250 mL glass beaker. Methylene blue solution (100 mL of 3×10^{-5} M) was added into beaker, and the solution was kept under magnetic stirring condition. The suspension from the reaction mixture was centrifuged at 7000 rpm and the resulted clear solutions were analyzed using UV–Visible spectrophotometer. We have performed similar studies using other dyes (other cationic, anionic and neutral dyes). In case of anionic or neutral dyes, there occurs almost no exchange reaction. APM moiety captures only cationic dyes selectivity.

2.4. Regeneration

Dye-APM colored composite materials were subjected to heat treatment for 12 h at 300 °C in an air oven. After the heat treatment, the samples become yellow i.e., YAPM. Thus the original material (yellow APM) was regenerated. DRS spectrum of this sample (after heating) was found to be similar to that of parent APM. This material was reused several times for dye removal through the same ion exchange reaction. We have tested the material for consecutive three cycles. After three cycles, the material was treated with conc. HNO_3 acid and its original activity has been observed to be restored, gratifyingly again it showed the retention of original band gap position.

3. Result and discussion

Solid APM is a well-known intricate polyoxometalate having giant molecular structure. We have presented the cation exchange property of APM and this property has been exploited for the removal of toxic dye molecules from wastewater for environmental abatement. Generally, transition metal oxides and sulfide were used as adsorbents or catalysts to remove organic waste from water by adsorption followed by combustion at relatively low temperature, photocatalytic reactions, *etc.* In this article, the removal of dyes through ion exchange property of YAPM as well as GAPM are discussed.

YAPM is a compound with UV light absorption capacity. Upon absorption of UV light (~365 nm) over a long time (3 days), the yellow

material is photoreduced to deep green compound in solid state [27] which is named as GAPM. The optical property of both the materials are investigated by DRS spectroscopy (Fig. 1). Broad absorption spectra were observed in both the cases. The peaks at 418 nm and 288 nm are found for the YAPM. Green APM (GAPM) exhibits peaks at 288 nm, 402 nm and with one extra band at 805 nm (Fig. 1a). In accordance with the UV–Visible spectral measurement, inter-band or excitonic transitions in YAPM and GAPM have also been observed. Theoretical expression for the determination of band gap of a semiconductor material is given by the following equation:

$$\alpha E_p = K\ (E_p - E_g)^{1/2}$$

where α is the absorption coefficient, K is a constant, E_p is the discrete photoenergy, and E_g is the band gap energy. A classical Tauc approach [29] is used to estimate the E_g of the material. The plot of $(\alpha E_p)^2$ versus E_p based on the direct transition and extrapolation of E_p at $\alpha = 0$ gives absorption edge energy which is band gap of the materials (Fig. 1b). From the plots, it is observed that the yellow material (YAPM) has band gap of 2.5 eV.

Remarkably, upon UV light exposure hoarding of electrons in the basket like structure of the yellow materials is readily observed as indicated by the position of band gap change (shown in Fig. 1b. Interestingly this observation complies with our reported EPR results [27] and also with an earlier report [30]. Conversely, this photochemical changeover was not detectable from common physical measurements like FTIR and powder XRD analysis (shown in Fig. S2 in Supporting Information). The dye exchange reaction does not affect the phase purity or crystal bond vibration as dyes remain bound onto the surface electrostatically of the robust anionic $[PMo_{12}O_{40}]^-$ moiety. Thus it may be said that the changeover took place only on the upper surface of the material by ion exchange. Presumably rigid skeletal dye moiety cannot invade into the phosphomolybdate skeleton reasonably. Photochemical color change from yellow YAPM to GAPM happens and this change is reversible under the UV light and or dark/ambient material storage conditions (Fig. 2).

This reversible color change is identified by UV–visible study in transmittance mode with time (shown in Fig. S3 in Supporting Information). Actually, the color change from green APM to yellow APM (reaction in the dark takes about 3 months) is extremely slow. That is why, we have shown the time course of UV–vis spectrum for the color change from yellow to green only.

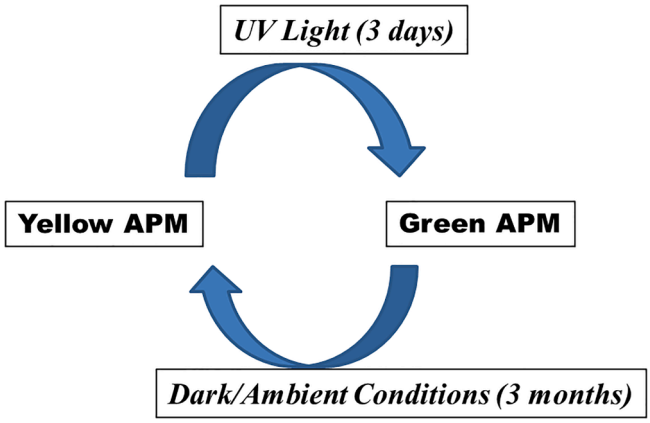


Fig. 2. Reversible conversion of yellow APM and green APM (GAPM).

After solid state photochemical reaction, there happens no change or distortion in morphology of APM i.e., anionic $[PMo_{12}O_{40}]^-$ moiety (investigated even by FESEM analysis) i.e. the yellow and green APM materials contain same shape and size which is shown in Fig. 3. This type of reversible photochromatic behavior between yellow APM and green APM (GAPM) is unusual and has been found with Ag-TiO_2 [31].

In aqueous solution of dye, solid APM was added and allowed for facile exchange reaction to happen by stirring using a magnetic stirrer. The dye-APM composite material settled down after waiting for 1 h or by centrifugation gives clear supernatant aqueous solution which is readily used for UV–visible studies.

We have monitored the exchange reaction by UV–visible spectroscopy at the band position of 662 nm of methylene blue (Fig. 4). With the progress of the reaction, the absorption value at 662 nm gradually decreases. We have performed the exchange reaction with different amount of yellow APM (0.1–0.2 g) and the spectral profiles as well as

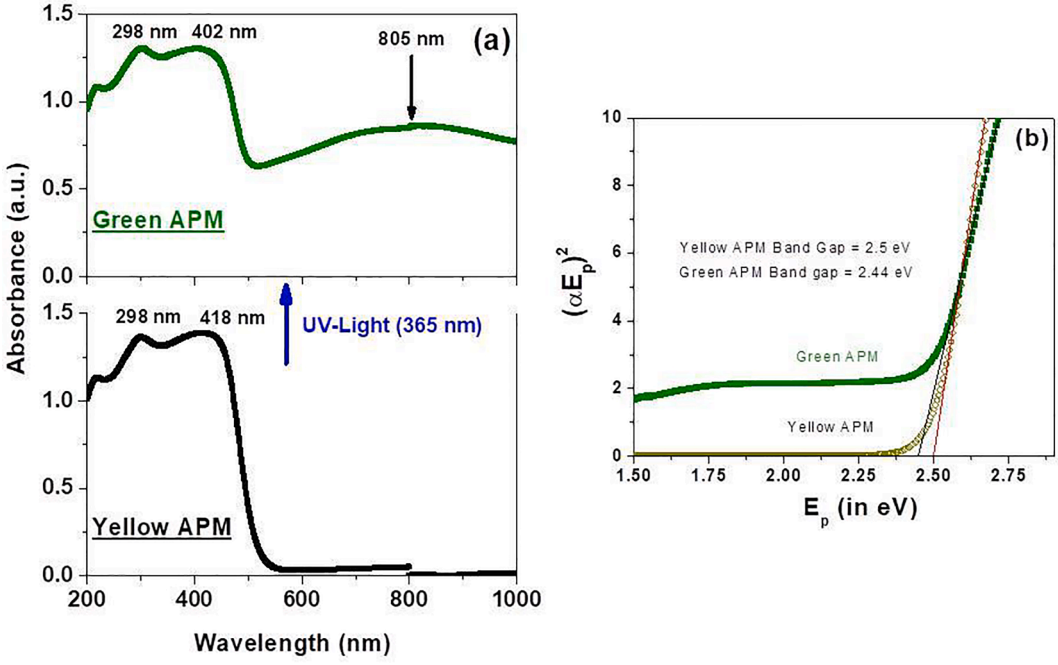


Fig. 1. DRS spectra for the formation of green APM from yellow APM under UV light exposure at 365 nm (a) and indication of the change in band gap while yellow APM changes to green APM (b).

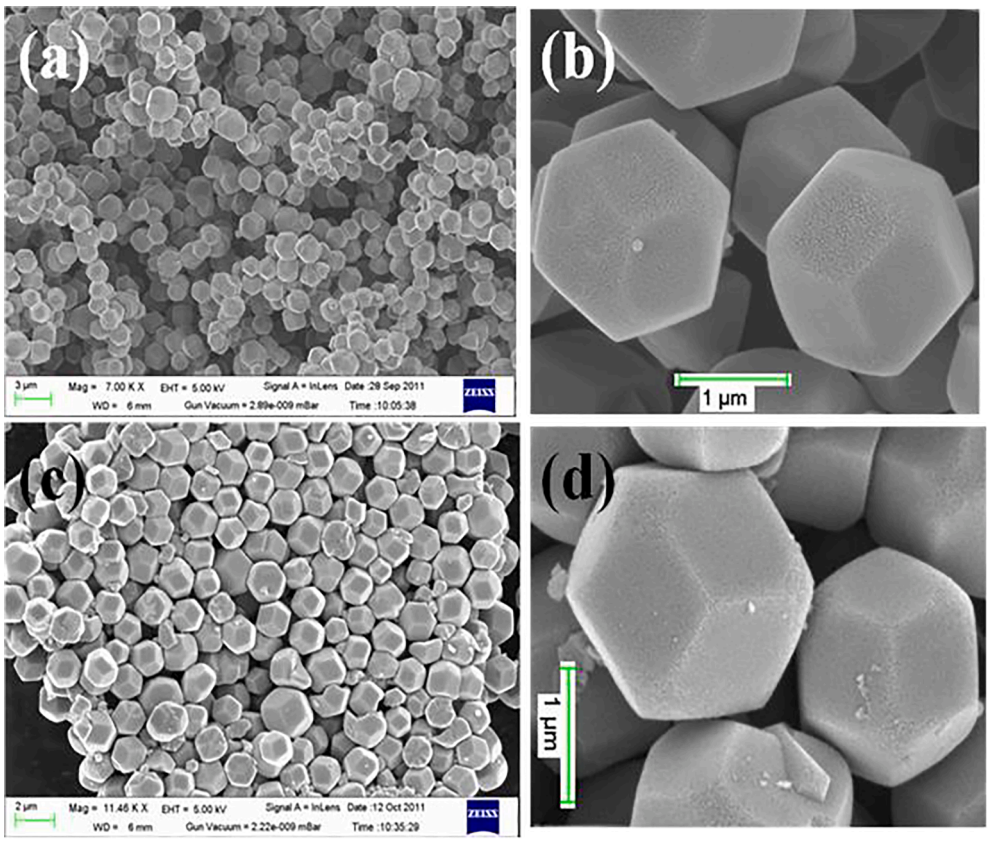


Fig. 3. FESEM image of Yellow APM (a and b) and UV light treated green APM (c and d).

time dependent color change are shown Fig. 4. A higher dose of yellow APM provides larger surface area, so the time of exchange reaction becomes shorter, and conversely, a lower amount of yellow APM sample takes longer time to complete the exchange reaction.

In similar way, other cationic dye molecules (such as rhodamine B, malachite green) are also separated from aqueous solutions and the corresponding dye exchange reactions have also been monitored again by UV–Visible study which is shown in Supporting Information (Fig. S4). After dye exchange reaction on APM, the color of the dye-APM composite changes to the corresponding color of dye which is shown in Fig. 5 by digital images.

Then the dye-APM composite material is heated at ~ 300 °C for 12 h. Hence, the bound dye molecule in APM matrix is completely decomposed. The decomposition process of organic dye is shown in Fig. 6b by TG analysis. From the TG analysis, at high temperature range (400–500 °C), it is observed that the host APM material undergoes partial decomposition like other molybdenum compounds [32]. That is way, we select 300 °C for our thermal reaction. The TG profile (at 400–500 °C range) is little bit different for the above material due to the temperature dependent variation in composition (APM a pure inorganic material and Dye-APM an organic–inorganic hybrid composite material) of the material. The colorful dye-APM composite material regains the original yellow APM color and gets ready for reuse for at least 3 cycles (shown in Fig. 6a).

During heating at temperature ~ 300 °C, the colored Dye-APM composite breaks down to gaseous molecules and leaves yellow APM as the decomposition product. We have used different cationic dye molecules as a model contaminant in water which contain alkyl amine as a functional group. It is expected that Dye-APM change to $(NH_4)_3PMo_{12}O_{40}$ at this elevated temperature and NH_4^+ ions are regenerated or come from nitrogenous part of the dye molecules. This was presumed from the dye adsorption studies with the decomposition product.

After repetitive use, the material loses its original adsorption activity. Consequently, the ion exchange time increases (shown in Fig. S5a–c of Supporting Information). Organic dye molecules upon heat treatment presumably generates small carbon nanoparticles which presumably get deposited on the active surfaces of YAPM. These carbon particles from the active sites of YAPM is easily removed by concentrated HNO_3 acid treatment which removes the carbon NPs from the APM surface. Carbon particles are oxidized by HNO_3 and thus the original activity of yellow APM is restored (shown in Fig. S5d of Supporting Information).

The exchange capacities of yellow and green APM materials were compared with other materials such as oxide matrix and commercial cation exchange resin (information is summarized in Table 1 of Supporting Information). The best result comes out from yellow and green APM compounds. One gram of yellow APM uptakes MB dye molecules from 70 mL of 10^{-3} M and the deep blue color of aqueous solution of methylene blue becomes colorless.

The dye exchange reaction of APM matrix with methylene blue was performed also in presence of different cations such as Na^+, Ca^{2+}, Mg^{2+} *etc.* These ions are present in sea water, lake water, pond water and tap water. An aliquot of MB solution was mixed with sea water, lake water, pond water and tap water separately, and the solutions were used for same exchange reaction by YAPM. The UV–Visible study for MB separation from different water system is shown in Fig. S6 Supporting Information. It is evident that when high concentration of alkali metal ions presents in the water sample, the duration of dye exchange reaction increases to a large extent. Precisely, the existing ions in water bodies become the inhibitor for the cationic dye adsorption and slow down the ion exchange reaction.

Dye exchange reaction has been studied using cationic, anionic and neutral dyes, and the mechanism of exchange reaction is shown in Scheme 1. The anionic and neutral dye molecules are not exchanged with the APM, and the corresponding step wise UV–visible study is shown in Fig. S7 of Supporting Information. APM can uptake selectively

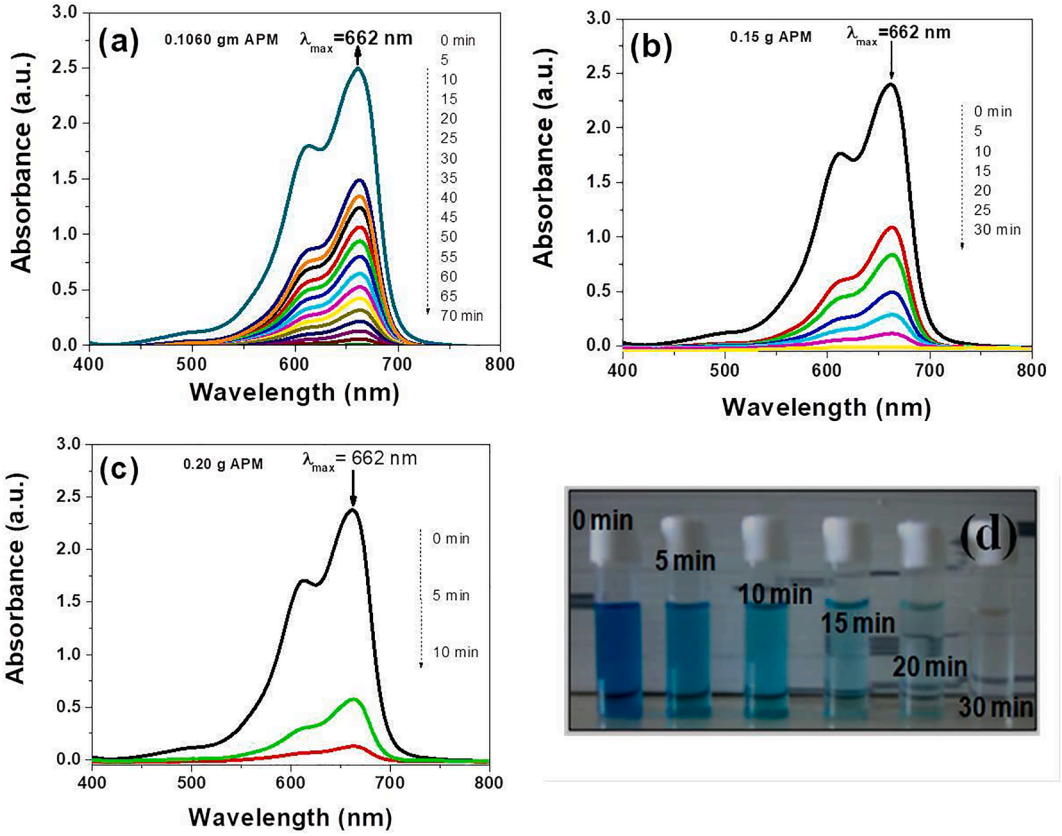


Fig. 4. UV–Visible study of methylene blue dye (5×10^{-5} M) exchange reaction at different time interval with (a) 0.10 g, (b) 0.15 g and (c) 0.2 g of yellow APM and a representative digital images showing methylene blue exchange reaction at different time interval.

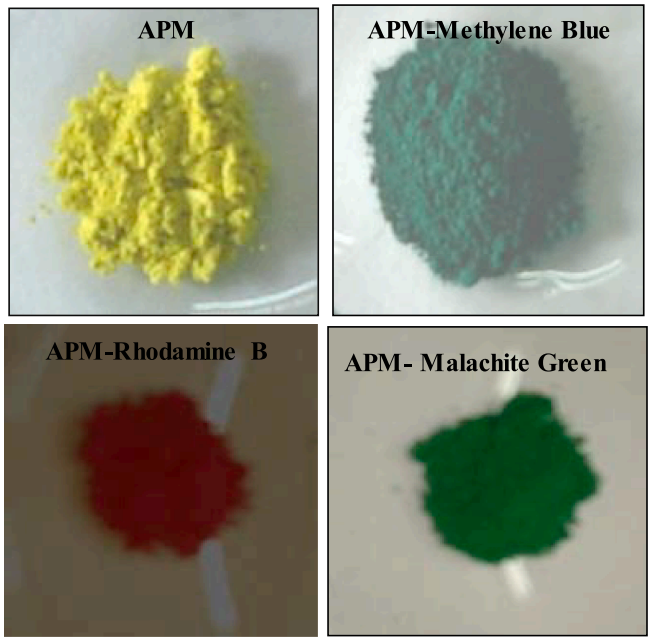


Fig. 5. Digital images of APM after dye exchange reaction: APM-methylene blue, APM-rhodamine B and APM-malachite green.

cationic dye molecules such as methylene blue, malachite green, rhodamine B, methyl violet (all are cationic dyes) effectively under ambient condition with different exchange/separation time. This relates to the skeletal size and electronic properties of the cationic dye molecules with amine funtionality. The dye molecules bearing rigid larger skeletal structure are slowly exchanged with smaller ammonium ion of APM. Anionic and neutral dyes after waiting a longer time, there occurs no exchange and slight decrease in absorbance values occur due to physisorption of dye molecule on APM moiety (Fig. S7 in Supporting Information). On the other hand, cationic dyes require much less time to complete the exchange reaction in comparison to anionic or neutral dyes. Thus physisorption is easily understandable due to the absence of electrostatic attraction. Additionally, the charge of the counter ion i.e. NH_4^+ in $[(NH_4)_3PMo_{12}O_{40}]$ plays an important role in the dye exchange reaction. The cationic dye molecules (dye^+) replace ammonium ion (NH_4^+) and form $[(NH_4)_m(dye)_nPMo_{12}O_{40}]$ ($n + m = 3$). The dye exchange reaction happened only on the outer surface not inside the APM microcrystal because of steric crowding and rigid dye skeleton. The interaction between cationic dye and APM is investigated by solid DRS analysis. The band position of cationic dye molecule is blue shifted in Dye–APM composite material. The DRS spectra of dye-APM composite materials are shown in Fig. S8 Supporting Information. This DRS analysis indicates that there exists an electrostatic interaction between the individual dye molecule and yellow APM matrix furnishing λ_{max} shifting of the dye molecule from the original value. In case of MB-APM composite, the band position of MB is shifted from 662 nm to 628 nm this hypsochromic shift indicates that there is significant electrostatic interaction between APM and MB. The electrostatic interaction is the main cause for the shifting of band position of MB. The exchange capacity values of a particular adsorbent vary from dye to dye depending on the size and electronic property of the dye skeleton as it is understandable. Upon UV light irradiation for a long time, the YAPM material changes to deep green APM (GAPM). The Keggin structure [in $(NH_4)_3PMo_{12}O_{40}$] inherits a special ability to accept one or several electrons, which remain delocalized within the Keggin basket without causing any structural deformation resembling a basket stored with electrons.

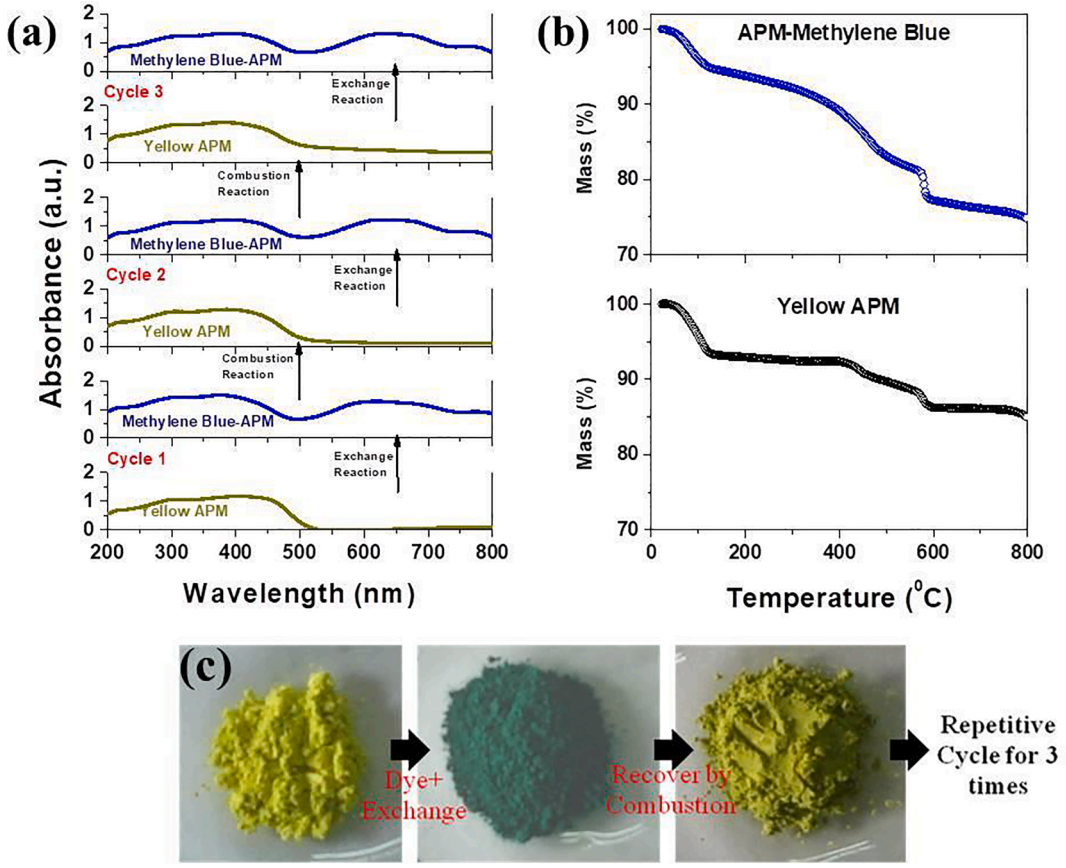
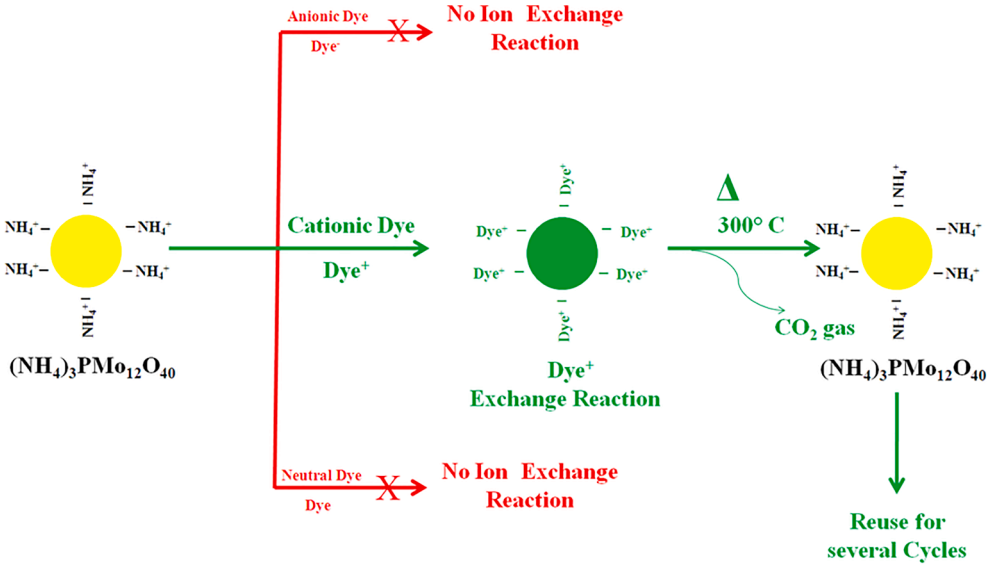


Fig. 6. Reusable of yellow APM (methylene blue dye exchange reaction) in three different cycles (a) and thermogravimetric analysis of methylene blue-APM and yellow APM (b) digital images of before and after methylene blue exchange on yellow APM and recovery of yellow APM by combustion method (c).



Scheme 1. Ion exchange mechanism and removal of dye molecules by yellow ammonium phosphomolybdate (APM) and regeneration of yellow APM.

The photoreduction happens only for the surface molecules of the solid ammonium phosphomolybdate. So it is expected that the ratio Mo^{5+}/Mo^{6+} will reach constancy at the surface. The lower oxidation state of Mo^{5+} is stabilized by H^+ ion. Our proposed mechanism has been shown in Fig. 7 where H_2O molecules are dissociated as $H^{\cdot}$ and $OH^{\cdot}$ radical by UV light [33]. The $H^{\cdot}$ takes part in the reaction that reduces Mo^{6+} of $(NH_4)_3PMo_{12}O_{40}$ to Mo^{+5} which is stabilized by H^+, a new exchangeable site for cationic dye molecules to interact.

The photochemical change i.e. YAPM to GAPM was monitored by UV visible spectroscopy in the transmittance mode (shown in Fig. S3 Supporting Information) through the accumulation of electrons.

The similar phenomenon, the UV light induced heterolysis of water (photo reduction), occur in the following semiconductor surfaces such as TiO_2 [34], ZnO [35], WO_3 [36], V_2O_5 [37] *etc.* and the materials change

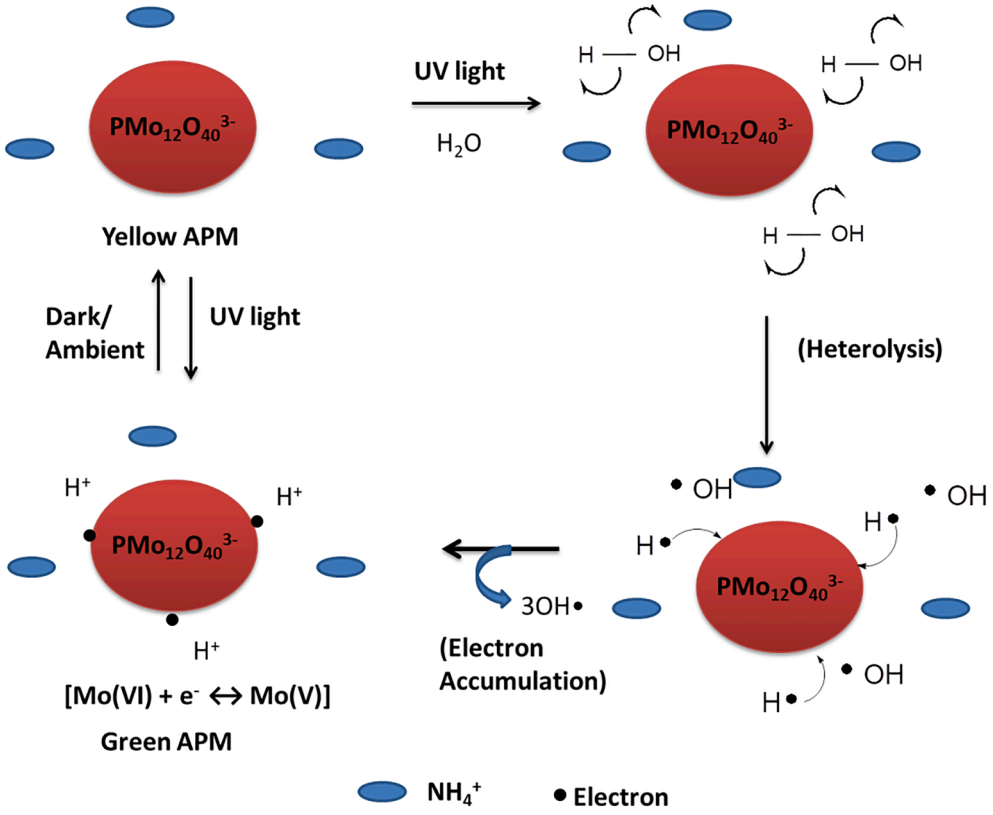


Fig. 7. Reversible pathways between yellow APM and green APM.

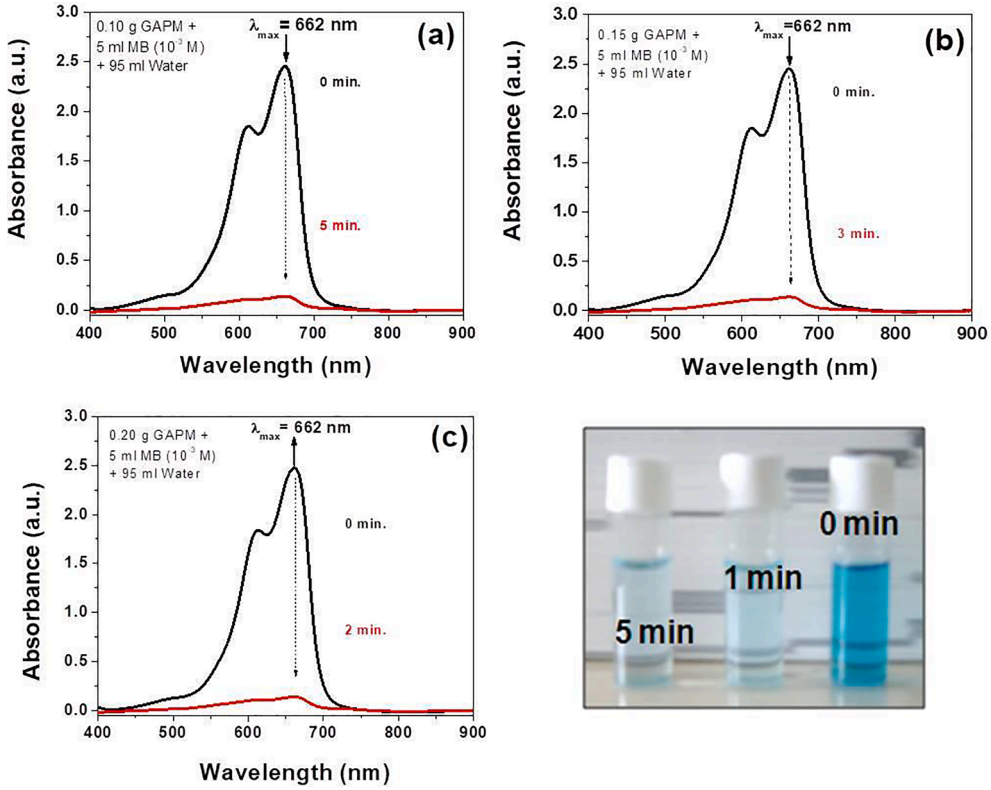


Fig. 8. UV–Visible study of methylene blue dye exchange reaction in different time gap with (a) 0.10 g, (b) 0.15 g and (c) 0.2 g green APM and digital image of methylene blue solution at different time of exchange reaction.

from hydrophobic materials to hydrophilic materials. In our experiment, we propose that yellow APM turns to green APM through capture of electrons from water molecules triggered by UV radiation [27,33]. The Keggin structure has strong ability to capture electron from hydrogen radical [33]. Generated hydroxyl radicals can react easily with water molecules or other species in the atmosphere.

As a result, after electron trapping by the Keggin ion GAPM is obtained reproducibly and the product can act as a better cationic dye scavenger. Within few minutes, the adsorbate dye is exchanged by GAPM through a stronger electrostatic force leaving aside a clear transparent water body at faster rate than yellow APM does. Influence of mass loading have been investigated which shows that dye exchange reaction becomes faster on enhancement of mass loading (Fig. 8a–c).

The dye exchange reaction with green APM (GAPM) is shown in Fig. 8. Thus the GAPM having hoarded electron shows better performances for the dye exchange reaction.

The dye exchange reaction by APM is studied at neutral pH condition and there occurs no deformation of the APM morphology after the cationic dye exchange reaction. It is worth mentioning that strong acidic waste water caused no hindrance for adsorption studies. Yellow ammonium phosphomolybdate (APM) material removes toxic cationic dye molecules from the wastewater of different sources (sea water, lake water, pond water and tap water) quantitatively and leaves no trace of adsorbate.

4. Conclusion

Ammonium phosphomolybdate (APM) collects electrons from photoactivation and becomes green APM. The green ammonium phosphomolybdate inherits basket full of electrons engendering increased cation affinity rendering better cation dye removal efficacy. Thus it becomes the novelty of UV activation process. Experiencing the promising applications of dye infested APM composite to bind simple molecules [34] and dielectric crossover and resistive switching performances of the pure material [35] from XRD, FTIR, Raman [36] and EPR [27] studies it is expected to have other future applications even for dye sensitized solar cell.

CRediT authorship contribution statement

Arun Kumar Sinha: Methodology, Writing - original draft, Investigation. **Anup Kumar Sasmal:** Writing - review & editing. **Anjali Pal:** Writing - review & editing. **Debasish Pal:** Writing - review & editing. **Tarasankar Pal:** Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jphotochem.2021.113427.

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Research Article

Oxidation of cyclohexene with hydrogen peroxide over nano-crystalline $Mn_xCe_{1-x}O_{2-\delta}$ catalyst

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The liquid-phase catalytic oxidation of cyclohexene to produce cyclohexenol and cyclohexenone directly was attempted using Mn ion substituted in ceria in acetonitrile solvent with 30% H_2O_2 as oxidant under atmospheric pressure. Structural studies by XRD show indication of ionically dispersed metal over ceria. Among all the catalysts studied, the $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ catalyst prepared by the solution combustion method has shown more activity (95.3% conversion with 98.7% selectivity) than others. The influences of the amount of Mn loading, temperature, time, the concentration of H_2O_2 and solvent have also been investigated. The enhancement of activity in Mn^{2+} ion substituted ceria as compared to other catalysts has been attributed to Mn–O–Ce ionic interaction in the combustion synthesized catalyst. Ionic substitution also helps to get an active stable catalyst with lower risk of Mn-leaching compared to the impregnated catalyst.

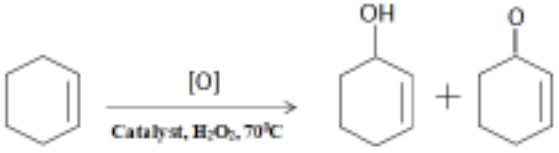
Key words: Oxidation, Cyclohexene, $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ catalyst, Ionic interaction

1. Introduction

One of the most important current topics of catalysis research has been to find an efficient catalyst for the selective oxidation of cyclohexene [1–6]. Oxidation of cyclohexene generally yields various products such as cyclohexenol, cyclohexenone, cyclohexene oxide and cyclohexene peroxide etc. [7]. Allylic oxidation of cyclohexene gives the products such as cyclohexenol and cyclohexenone as major products along with cyclohexene oxide as the minor product, indicating the involvement of Fenton-type oxidation reactions [8]. But when the oxidation occurred on the double bond, it gives cyclohexene oxide or epoxide as major products along with cyclohexene diols as minor product [4]. Major formation of the allylic oxidation products show the preferential attack of the activated C–H bond over the C=C bond [9]. However, the selectivity towards these products depends on various parameters like reaction conditions, central metal ion, solvent, oxidizing agent, nature of the catalyst etc. [10–14]. Previously oxidation of cyclohexene oxidation was carried out by using inorganic oxidants such as permanganate and chromium oxide [15]. Among the oxidizing agents hydrogen peroxide was chosen as a clean oxidizing agent, since it is inexpensive, environmentally friendly and generates water as byproduct [16].

Various new efficient catalytic systems have been designed and developed for the oxidation of cyclohexene with hydrogen peroxide as an oxidant. Among the catalytic studies, transition metal complexes are used as very effective homogeneous catalysts for this oxidation reaction [17–20]. But decomposition or degradation is one of the major problems in the application of homogeneous transition metal complexes [21, 22]. On the other hand separation of the products from the reaction mixture is also a major problem in case of homogeneous catalysts. Decrease of degradation of homogeneous catalysts have been achieved by the methods such as covalent anchorage on polymers [23], using inorganic oxide as supports [24–26] and also entrapped inside a porous oxide framework [27]. However, deactivation of the metal based catalysts due to leaching of active metal from the catalyst is still challenging [28]. This can be controlled by adopting specific preparation procedure and incorporating with other supports [29].

Here we show that $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ prepared by a single step solution combustion method has a much higher selective oxidation activity towards cyclohexene to cyclohexenol and cyclohexenone than other catalysts at 70 °C in acetonitrile solvent and atmospheric pressure (Scheme 1).



Scheme 1. Catalytic oxidation of Cyclohexene

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2. Materials and Methods

2.1 Preparation of catalyst

We have synthesized the catalysts by single step solution combustion method in an open muffle furnace kept in a fume hood with exhaust by the combustion of the corresponding metal nitrate salts with oxalyl dihydrazide [$C_2H_6N_4O_2$(ODH)] as the fuel. Oxalyl dihydrazide was prepared by the dropwise addition of diethyl oxalate ($C_2H_6N_4O_2$, Sisco Research Laboratories Pvt. Ltd., 99%) to ice-cooled aqueous solution of hydrazine hydrate ($N_2H_4.2H_2O$, Qualizens Fine Chemicals, 99%) as reported in [30]. Solution combustion synthesis (SCS) for the preparation of $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ involves combustion of the metal salts $(NH_4)_2Ce(NO_3)_6$, $Mn(NO_3)_2$,$3H_2O$ with ODH, taken in a molar ratio 0.90 : 0.10 : 2.26, at the temperature of ignition of the redox mixture (∼350 °C). In a typical preparation, 5 g of $(NH_4)_2Ce(NO_3)_6$ (Loba Chemie, 99%), 1.145 mL 10% $Mn(NO_3)_2.3H_2O$ (Merck India, 99%) solution and 2.5122 g of ODH are dissolved in 30 mL of double distilled water in a borosilicate dish. The solution is then transferred to the preheated muffle furnace maintained at ∼350 °C. Initially the solution boils with frothing and foaming followed by complete dehydration when the surface gets ignited and burns with a flame yielding a voluminous solid product within a minute. We have also prepared $Mn_xCe_{1-x}O_{2-\delta}$ (x = 0.03, 0.07, 0.10 and 0.15) catalysts in a similar manner.

In order for comparison, we have also prepared $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ by the incipient wetness impregnation (IWI) methods. For the preparation of the impregnated catalyst, the support (combustion made CeO_2) was first dried and then impregnated with an appropriate volume of the aqueous solution of manganese nitrate, corresponding to the support pore volume. The sample was then dried overnight at 100 °C, crushed and calcined at 500 °C for 3 h in air to get the catalyst.

2.2 Characterization of catalysts

The synthesized materials have been characterized by XRD. X-ray powder diffraction patterns were collected in a Rigaku diffractometer fitted with a horizontal goniometer mounted on a rotating anode. These data were recorded at 4 kW (40 kV, 100 mA) at 1° min^{-1} with a step size of 0.02° in the range 20 to 80 degrees. The rotating anode has Cu anode with effective wavelength of 1.5418 Å. There is a diffracted beam monochormator (Graphite crystal) which takes care of K_β lines and fluorescence.

2.3 Catalytic test

The oxidation of cyclohexene by H_2O_2 was carried out in the temperature range RT−80 °C at atmospheric pressure. In a typical experiment, 50 mg of catalyst was added to a liquid mixture containing 10 mL of acetonitrile, 2.45 mL of 30 wt% H_2O_2 (24 mmol) and 0.865 mL of cyclohexene (8 mmol) in a 250 mL two-necked round bottomed flask. For uniform mixing, the contents were stirred continuously during the course of reaction by a magnetic stirrer. The reaction system consisted of two liquid phases—an organic phase containing cyclohexene and acetonitrile, and an aqueous phase containing acetonitrile and 30% H_2O_2.

The reaction compositions were analyzed using a gas chromatograph (Nucon 5765, New Delhi) using a fused silica capillary column (EC5) of 30 m × 0.25 mm × 0.25 μm film thickness from Alltech and equipped with a FID detector. The injector and detector temperatures were 220 °C and 240 °C. The initial and final column temperatures were 110 °C and 150 °C, respectively with a temperature programmed rate of 80 °C min^{-1}. The quantitative analysis was done by standard sample injection.

Catalyst recycling was carried over the most active SCS and its corresponding IWI catalysts only. After each experiment, the reaction mixture was allowed to settle. Then the solution was filtered and the solid residue was washed thoroughly with the solvent. After washing, the solid residue was dried at 100 °C for overnight. This was used as catalyst for the next cycles to check the recycling ability of the catalysts.

3. Results and Discussion

3.1 XRD studies

Powder XRD patterns of SCS made $Mn_xCe_{1-x}O_{2-\delta}$ catalysts as well as the IWI catalyst are shown in Figure 1. All the diffraction lines can be indexed to the fluorite structure of Ceria (Fm3m) only [31] [JCPDS card no. 34–0394]. Thus other than the solid solutions phase no other peak(s) due to manganese oxide or metal are detected in XRD. This again points to Mn^{2+} ion substitution for Ce^{4+} in CeO_2 matrix. The corresponding IWI catalyst in which MnO is dispersed over ceria also shows no peak due to this phase in the XRD pattern. Thus, MnO crystallites in the IWI catalyst are so finely distributed over ceria that they escape XRD analysis. A much slower scan might have shed more light on the evolution of different Mn-phase(s).

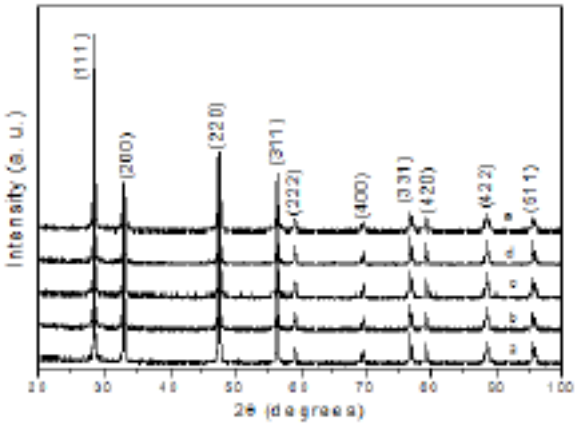

Figure 1. XRD patterns of (a) $Mn_{0.05}Ce_{0.95}O_{2-\delta}$, (b) $Mn_{0.07}Ce_{0.93}O_{2-\delta}$, (c) $Mn_{0.10}Ce_{0.90}O_{2-\delta}$, (d) $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ aged and (e) $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ IWI

4. Activity studies

4.1 Screening of catalysts

Table 1 lists cyclohexene oxidation activities of all the catalysts investigated here. The $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ catalyst shows much higher reactivity ($\sim$ 95% conversion) and selectivity (99%) to cyclohexenol and cyclohexenone than the other catalysts after 3 h. The effect of varying catalyst loading of on the reaction is also investigated (Table 1). The enhanced oxidation activity over $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ catalyst indicates promoting effect of ceria in the combustion synthesized catalyst.

Table 1: Cyclohexene oxidation activities of different catalyst.

Catalyst	Amt. of cat. (mg)	Conv. (%)	Products (%)			Sel. (%)
			Cyclohexenol	Cyclohexenone	By-product*	
$Mn_{0.03}Ce_{0.97}O_{2-\delta}$	50	86.4	26.6	53.9	5.9	93.2
$Mn_{0.05}Ce_{0.95}O_{2-\delta}$	50	95.3	26.8	67.3	1.2	98.7
$Mn_{0.07}Ce_{0.93}O_{2-\delta}$	50	95.6	25.9	65.2	4.5	95.3
$Mn_{0.10}Ce_{0.90}O_{2-\delta}$	50	88.3	23.7	59.1	5.5	93.8
$Mn_{0.15}Ce_{0.98}O_{2-\delta}$	50	86.4	21.5	53.5	13.3	86.8
$Mn_{0.05}Ce_{0.95}O_{2-\delta}$	30	92.3	26.1	63.8	2.4	97.4
$Mn_{0.05}Ce_{0.95}O_{2-\delta}$	70	93.2	25.5	63.6	4.1	95.6

Reaction condition: 0.865 mL cyclohexene + 10 mL MeCN + 2.450 mL H_2O_2 + 70 °C + 3 h;

*byproducts are mainly cyclohexene oxide and a little bit of cyclohexenediols.

The $Mn_{0.03}Ce_{0.97}O_{2-\delta}$ sample and 30 mg $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ catalyst are also found to be already very active. But a conversion of 95.3% with 98.7% selectivity was observed for 50 mg $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ catalyst which is higher than other catalysts. Although the 7 atom% metal loaded catalyst ($Mn_{0.07}Ce_{0.93}O_{2-\delta}$) showed slightly higher conversion than the $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ catalyst, the latter showed higher selectivity than the former. Any further increase in manganese content and amount of catalyst caused a decrease in the cyclohexenol and cyclohexenone formation. The lower activity of higher loaded (> 5 atom%) catalysts indicate that as manganese loading increased, percentage of MnO phase increased and manganese dispersion decreased resulting in reduced activity and selectivity. Hence we chose $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ as the best formulation for further investigation.

4.2 Temperature effect

The effect of temperature on the oxidation of cyclohexene was studied by varying the temperature from 35 °C to 80 °C over the catalyst (50 mg) with other parameters kept constant and the results are shown in Figure 2. Selection of this particular temperature range is due to the fact that at higher temperatures the decomposition of H_2O_2 predominates [32]. At room temperature (35 °C)

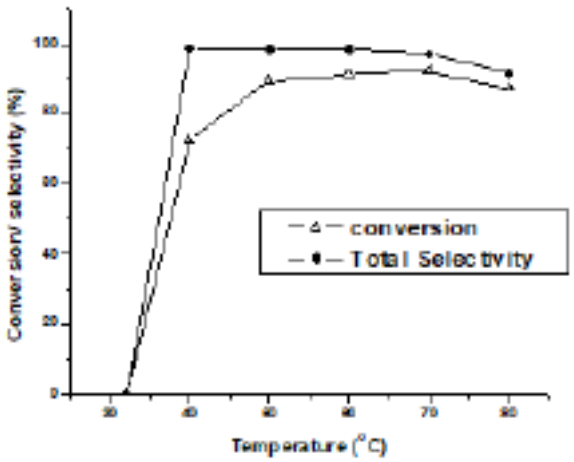

Figure 2. Conversion of cyclohexene/selectivity of cyclohexenol and cyclohexenone as a function of temperature over $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ catalyst. Reaction condition: 50 mg catalyst + 0.865 mL cyclohexene + 10 mL MeCN + 2.450 mL H_2O_2 + 3 h.

cyclohexene was not oxidized, showing no reactivity of the catalyst. But an increase of the reaction temperature by just 5 °C leads to a conversion of $\sim$ 31% with total selectivity of $\sim$ 90% to cyclohexenol and cyclohexenone. Further 10 °C raise in temperature increases the conversion ($\sim$ 80%) and selectivity remains similar. The conversion is maximum (95%) between 60–70 °C with $\sim$ 99%

selectivity. It decreases marginally to ∼ 88% when reaction temperature was increased to 80 °C. The above data indicates that the competition between the products and byproduct occurs above 70 °C. Hence, the reaction temperature higher than this optimum temperature is in favour of byproduct formation in addition to the self-decomposition of hydrogen peroxide resulting to a relatively lower conversion. Thus 70 °C has been chosen as the most suitable temperature for the selective oxidation of cyclohexene under our reaction conditions.

4.3 Effect of reaction time

The cyclohexene conversion over $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ as a function of reaction time at 70 °C is presented in Figure 3. The oxidation starts at ∼ 30 min over the catalyst, progresses linearly to ∼ 80% upto 120 min and maximum activity (∼ 95% conversion) and selectivity (99%) is reached beyond ∼ 180 min. This is why we chose 3 h as reaction time in our studies.

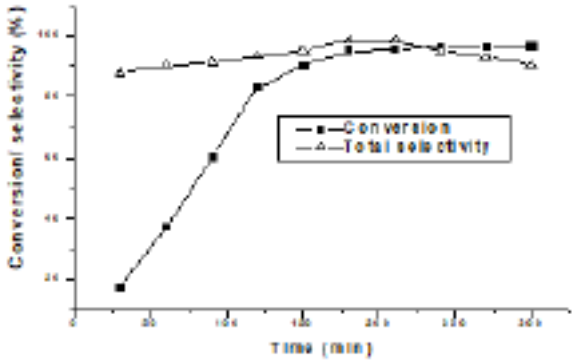

Figure 3. Conversion of cyclohexene/selectivity of cyclohexenol and cyclohexenone as a function of time over $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ catalyst. Reaction condition: 50 mg catalyst + 0.865 mL cyclohexene + 10 mL MeCN + 2.450 mL H_2O_2 + 70 °C.

4.4 Influence of H_2O_2 concentration

Cyclohexene oxidation was carried out by adding H_2O_2 to the reaction mixture in one lot at the reaction temperature. To study the effect of varying the amount of H_2O_2, the reaction was carried out with 50 mg of catalyst and the amounts of H_2O_2 from 8 mmol to 40 mmol while keeping other conditions unchanged. The results are shown in Figure 4. In absence of H_2O_2, cyclohexene was not oxidized which indicates that the catalyst cannot oxidize cyclohexene in presence of air (O_2) only. Percentage conversion and total selectivity for cyclohexene oxidation reaches a maximum value with 24 mmol of H_2O_2 and then these starts decreasing. The distribution of allylic oxidation products shows the same trend but the cyclohexene oxide percentage shows a gradual increase [4]. Even though the theoretical molar ratio of cyclohexene to H_2O_2 for the oxidation reaction is 1 : 1 and concentration of H_2O_2 was 8 mmol, here the results show that H_2O_2 needed was triple its stoichiometry. This can result from the fact that not all the H_2O_2 can take part in the oxidation due to its unavoidable self-decomposition under the reaction conditions [33].

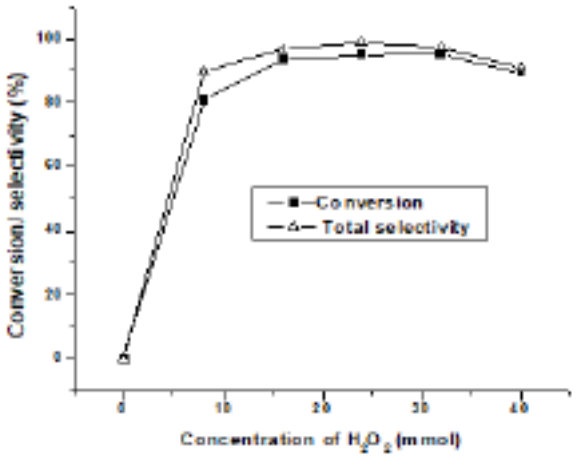

Figure 4. Conversion of cyclohexene/selectivity of cyclohexenol and cyclohexenone as a function of H_2O_2 concentration over $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ catalyst. Reaction condition: 50 mg catalyst + 0.865 mL cyclohexene + 10 mL MeCN + 70 °C + 3 h.

4.5 Effect of solvent

The cyclohexene oxidation was carried out using various solvents such as acetonitrile, methanol, ethanol and toluene over $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ catalyst and the results are presented in Figure 5. Acetonitrile is found to be the best solvent for cyclohexene oxidation with highest conversion of 95.3% and selectivity of 98.7%. Toluene showed lowest cyclohexene conversion (70%) but the selectivity (93%) was very high. Using ethanol or methanol as a solvent, comparatively lower conversion is observed.

In this study, it is believed that the solvent acetonitrile acted as a 'media' serving homogeneity for the liquid phase(s). Cyclohexene and hydrogen peroxide are both soluble in acetonitrile and the reaction products; viz., cyclohexenol and cyclohexenone are not only soluble in the reaction mixture but also can be displaced from the surface of catalyst as they are formed. Acetonitrile, an aprotic solvent, can also activate H_2O_2.

The $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ prepared by IWI method ($Mn_{0.05}Ce_{0.95}O_{2-\delta}$ IWI) gives a conversion of ∼ 81% with ∼ 92% selectivity than the same catalyst prepared by SCS method. These values are quite lower compared to those of the SCS catalyst.

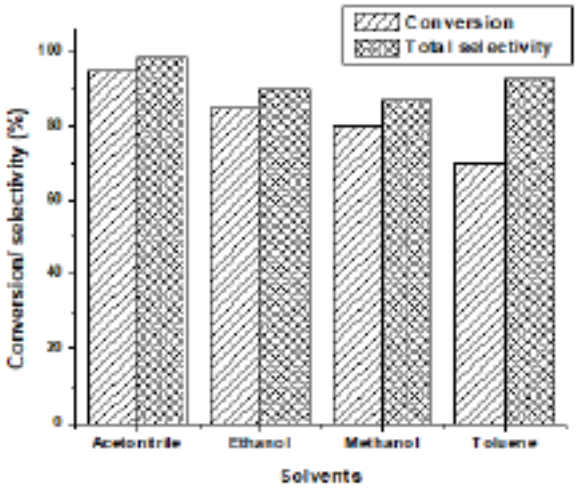

Figure 5. Solvent effects on cyclohexene oxidation activities over $Mn_{0.05}Ce_{0.95}O_{2-\delta}$. Reaction condition: 50 mg catalyst + 0.865 mL cyclohexene + 2.450 mL H_2O_2 + 70 °C + 3 h.

To check the stability and recycling ability of the SCS catalyst, the used catalyst was separated from the reaction mixture and dried in air at 110 °C, and then oxidation experiments were performed under the typical reaction conditions used here. The results show that the $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ catalyst maintains its activity after three consecutive cycles without any appreciable loss of conversion and selectivity.

The result of ageing experiments over both SCS and IWI catalysts show a decreasing trend for the IWI catalyst, whereas the SCS catalyst more or less maintains its activity (Figure 6). The loss of activity of the IWI catalyst during ageing is likely due to leaching of MnO from the catalyst.

The different catalytic activity of SCS and IWI catalysts is related to phase composition of the support oxide. In case of $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ made via combustion route, there is no MnO phase (as revealed from XRD study) and it shows better catalytic behavior than the corresponding IWI catalyst that contains very finely distributed MnO over ceria. Thus, Mn^{2+} in the ceria lattice sites (as in $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ catalyst) is more active than MnO (as in $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ IWI catalyst) towards cyclohexene oxidation. This higher activity of combustion synthesized catalyst compared to the impregnated catalyst can then be attributed to Mn–O–Ce ionic interaction in the $Mn_xCe_{1-x}O_{2-\delta}$ catalyst. The loss of activity of IWI catalyst during ageing is most likely due to leaching of MnO from the catalyst. The SCS catalyst contains Mn^{2+} ion sites in the ceria lattice which is difficult to be leached out from the catalyst surface and hence its activity remains unaltered during ageing in the reaction atmosphere.

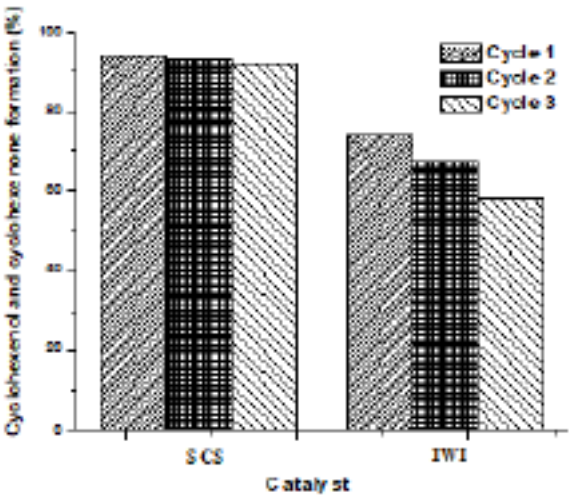

Figure 6. Effect of cycling on cyclohexene oxidation activities of $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ catalyst prepared via (a) solution combustion (SCS), (b) impregnation (IWI). Reaction condition: 50 mg catalyst + 0.865 mL cyclohexene + 2.450 mL H_2O_2 + 10 mL MeCN + 70 °C + 3 h.

Cyclohexene oxidation generally proceeds through a radical pathway in presence of a peroxide oxidant, in which the homolytic cleavage of the oxidant is involved [8,34]. To examine the mechanism involved in the present case, we performed the oxidation in presence of a radical scavenger (quinone). The radical scavenger was added to the reaction mixture after 1 h of reaction and the progress of reaction was monitored. Cyclohexene oxidation was found to be totally stopped after addition of the scavenger. Therefore, we believe that cyclohexene oxidation over the reported catalysts in this study proceeds via a radical mechanism [35]. Hydroxide radical is first produced that subsequently abstracts hydrogen from the reactant to produce cyclohexenyl free radical. This radical is then trapped by O_2 and/or combine with hydroperoxide radical to form cyclohexenyl peroxide intermediate which eventually leads to the formation of cyclohexenol and cyclohexenone.

5. Conclusions

In this study, solution combustion synthesized single phase $Mn_{0.05}Ce_{0.95}O_{2-\delta}$ is shown to be a highly efficient catalyst for the oxidation of cyclohexene in acetonitrile solvent at 70 °C than the other catalysts investigated. The conversion of cyclcohexene achieved over this catalyst is $\sim$ 95% and the selectivity of cyclohexenol and cyclohexenone was almost $\sim$ 99%. XRD study indicates ionic substitution of manganese in ceria. The high activity can thus be attributed to active Mn^{2+} ion sites and Mn–O–Ce ionic interaction in the $Mn_xCe_{1-x}O_{2-\delta}$ catalyst. Since Mn is incorporated as Mn^{2+} ion in the structure of ceria, the possibility of its leaching is diminished. The ageing and recycling experiments confirm this since there was no loss of activity of the SCS catalyst due to these treatments. On the other hand, the activity of the IWI catalyst is lower due to presence of MnO crystallites over the ceria support where leaching of the active phase is easier as observed in the ageing experiment where a decreasing activity trend is observed.

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Conflict of interest

The authors have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Synthesis, characterization of one Cr (III) complex: An efficient chemosensor for Cr (III) ions and designing of a molecular logic gate

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ABSTRACT

A simple spectrophotometric based method for the detection of both the trivalent and hexa valent forms of chromium using 3-amino-2-pyrazine carboxylic acid (APC) is reported. Using APC as a ligand with $Cr(NO_3)_3.6H_2O$ a new mononuclear complex has also been synthesized and systematically characterized by elemental analysis and IR studies. Single crystal X-ray diffraction analysis reveals octahedral-type geometry with N_3O_3 coordination environments from the ligand. The optical sensing properties of APC for metal ions were enlightened by UV-Vis study. This sensor displays high selectivity and sensitivity towards Cr^{3+} in methanol. Interestingly the ligand displayed enhancement due to chelation with $Cr^{3+}$and quenching while interacting with Cr^{6+} ions. Complete opposite spectroscopic behavior helps us to identify the oxidation state of chromium.The logical response of the molecular AND gate is realized and as the APC can efficiently sense Cr^{3+} in solitary as well as crowded conditions, presence of M^{+n} ions would not have any effect to the logic functioning of the molecular AND gate.

1. Introduction

Molecular logic gate is one of the research interests in chemistry for efficient use in information technology, a significant development in the molecular sensing investigations to proposed molecular logic devices, such as logic gates [1–3], molecular keypad locks [4], information storage devices [5] and so on. These logic instruments are supposed to transfer the molecular level information to the obtained optical signals [6]. The first AND logic gate was developed using optical signals by de Silva et al. [7]. Since then, different chemical systems have been developed for various logic functions such as OR, AND, NOT [8]. Designing of molecular logic devices has earned an enormous attention from different research groups across the globe [9–11]. Besides just Boolean functioning, molecular logic gates have shown their potential applicability in diverse fields including ion sensing, [12] neuronal imaging [13] etc.

On the other hand, the carcinogenicity of chromate dust has been documented since the late 19th century. Chromium and its high valent oxoanions result from extensive anthropogenic uses such as leather tanning, electroplating, catalyst for halogenations, alkylation, catalytic cracking of hydrocarbons, in steel industry, printing, cement, mortar, wood preservative etc [14,15]. Compared to other oxo-species pollutants, hexavalent oxoanions, namely chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$), are highly soluble in water and therefore can contaminate into water easily [16]. Water pollution is becoming a major concern to occupational and environmental health and needs to be addressed in recent times. These species may also come into drinking water supply systems from the corrosion inhibitors used in water pipelines [17]. The World Health Organization recommends a maximum allowable concentration of 0.05 mg/L of Cr(VI) in drinking water. Because of its comparatively smaller size, Cr(VI) is genotoxic, hemotoxic and carcinogenic and its high oxidation potential makes it capable to enter through the biological cell membranes easily. On the other hand, Cr(III) is less toxic and less mobile and it is an essential trace mineral for the biological systems. Nonetheless, it can oxidize to carcinogenic Cr(VI) easily by manganese present in the soil and Cr(III) will also show the detrimental health effects if exposed to it for a long time [18]. Cr(VI) is reduced to Cr(III) by various intracellular reducing agents and then Cr(III) binds to DNA through guanine N7 and the adjacent phosphate backbone [19]. Hence, discriminative detection and segregation of the targeted trace level toxic ions from drinking water, physiological or environmental sources is a subject of great importance as potential biomarkers in contemporary environmental research [20]. Al-

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though there are many conventional instrument-assisted approach like AAS, ICP-AES/MS, HPLC, CPEs etc. but there are several important disadvantages such as high cost, high time consumption and necessity of sophisticated instrumentations [21]. These can be avoided by the use of much simpler methods with rapid response time, high sensitivity and operational simplicity such as spectrophotometric approach. So, there is a growing demand to develop reusable, eco-friendly probes for easy capture of Cr(VI) oxoanions from waste materials selectively and accurately. In the last few years research activities concerning to the organic moiety and MOF [22–24] as luminescent probes have rapidly developed but only a few reports have showed estimation of Cr-species without pre-separation of other individuals by fluorescence quenching techniques and differentiation between two oxidation states of chromium by using one simple organic moiety are still rare in the toxicological studies.

Small molecules are easier to determine as well as manipulate their properties like geometry, coordination or donor strength etc. than surface-graphed or polymer materials. Thus, small molecules are chosen in order to explore underlying efficient sensing phenomena [25]. Addressing these ideas here we report the use of 3-amino-2-pyrazine carboxylic acid (APC) as an efficient spectrophotometric probe that can selectively differentiate between two oxidation states of chromium. APC was chosen asthis has the effective binding sites with nitrogen of amine group and oxygen atom at ortho position, which enhances the coordination ability toward different metal ions along with its ability to promote Intramolecular charge transfer (ICT). The selected ligand has interesting binding components, may form interesting supramolecular architecture, so can acts sensor to the metal ions (Scheme I), causing a change that can be detected by the UV-vis spectroscopy.

As we are interested to find low cost and easy method to sense chromium ion selectively, so this probe may fulfill the requirements and offers a rapid sensitivity for the separation of chromium ion in presence of the other metal ions. This determination is based on the ion-association complex between the organic moiety and Cr(III) ions.The binding of the probe with Cr(III) is firmly established by the SCXRD technique and supported by UV-Vis spectroscopy. Here, two-input-single-output AND and PASS 0 gates are designed considering the opto-chemical responses generated from the ligand (APC) on interaction with different metal ions. Further, the different logic responses are exploited for the selective and efficient sensing of Cr(III) ions in a smart mannerutilizing the ligand, APC.

2. Materials and methods

2.1. Materials

All required chemicals and solvents used for the synthesis were of analytical grade and purchased from Sigma-Aldrich.

2.2. Synthesis of $[(C_5H_4N_3O_2)_3Cr]$

10 mL methanolic solution of 3-amino-2-pyrazine carboxylic acid (0.13 g) was added to a 15 mL methanolic solution of $Cr(NO_3)_3{\cdot}6H_2O$ (0.040 g) with continuous stirring. The resulting red colored solution was heated to 120 °C for 6 h in a Teflon flask. The flask was cooled at room temperature. After 3 days orange colored hexagonal crystals suitable for X-ray analysis were collected by filtration and washed with methanol. Yield: 70%. Anal. Calcd (%) for $[C_{15}H_{12}CrN_9O_6]$: C, 38.62; H, 2.59; N, 27.04. Found (%):C, 38.68; H, 2.61; N,26.97;IR (KBr, cm^{-1}): ν O-H3338 cm^{-1}; ν N-H 3456 cm^{-1}; ν C-N 1317 cm^{-1}; UV–vis, λ_{max} (nm, CH_3OH): 244(π–π^*); 341 (n–π^*).

Table 1
Crystal data and refinement parameters of complex $[(C_5H_4N_3O_2)_3Cr]$.

	Complex
Empirical formula	$C_{15}H_{12}CrN_9O_6$
Formula weight	466.34
Temperature/K	294
Crystal system	Monoclinic
Space group	C 2/c
a/Å	29.937(3)
b/Å	8.2626(9)
c/Å	14.4417(15)
α/°	90
β/°	93.196(4)
γ/°	90
Volume/Å^3	3566.8(7)
Z	8
ρ_{calc}g/cm^3	1.737
μ/mm^{-1}	0.702
F(000)	1896
Crystal size/mm^3	0.11 × 0.09 × 0.03
Radiation	MoKα (λ = 0.71073)
Θ range for data collection/°	2.56–25.5
Index ranges	$-36 \le h \le 36$, $-10 \le k \le 10$, $-17 \le l \le 17$
Reflections collected	35,241
Independent reflections	3309
Data/restraints/parameters	3309/0/304
Goodness-of-fit on F^2	1.059
Final R indexes [I>=2σ (I)]	R_1 = 0.0349 wR_2 = 0.0907

$w = 1/[\sigma^2(F_o^2)+(0.0331P)^2 + 2.2179P]$ where $P=(F_o^2+2F_c^2)/3$.

2.2. Physical measurements

FT- IR spectra were collected by using a Perkin-Elmer FT-IR spectrophotometer in KBr pellets (4000–400cm^{-1}). Absorption spectra were recorded on Perkin-Elmer Lambda-35 spectrophotometer using a 1.0cmquartz cell.

2.3. X-ray crystallography

2.3.1. X-ray diffraction data of $[(C_5H_4N_3O_2)_3Cr]$

Diffraction data were collected at 294 K on a Bruker D8 VENTURE CCD diffractometer using graphite monochromated Mo-Kα radiation (λ = 0.71,073 Å). APEX3 and SAINT programs of the Brucker 2016 package [26] were used for data collection, data reduction and cell refinement. The structure was solved by SHELXT [27] and refined by full-matrix least squares based on F^2 with SHELXL-2018/3 [28]. All non-hydrogen atoms were refined anisotropically. The amine H atoms were located in a difference Fourier map and refined freely. All other H atoms were placed geometrically and refined using a riding atom approximation, with $C - H$ = 0.93 Å, and with $U_{iso}(H) = 1.2U_{eq}(C)$.

Crystallographic data are summarized in Table 1. Selected bond lengths and angles are given in Table 2. The molecular graphics and crystallographic illustrations were produced using the Ortep-3 [29] and SCHAKAL-99 [30] programs.

3. Results and discussion

3.1. Description of the crystal structure for complex $[(C_5H_4N_3O_2)_3Cr]$ (1)

The asymmetric unit of **1** contains a mononuclear complex molecule including three 3-amino-2-pyrazinecarboxylato anions and one Cr^{3+} cation (Fig. 1). Each anion acts as a bidentate ligand coordinating through a carboxylic O atom and a pyrazine N atom to give a *mer*-CrN_3O_3 octahedral configuration. The coordination geometry around the metal deviates slightly from ideal octahedral as evidenced by the *trans*-oriented coordination angles varying from 169.83(9) to 173.14(8) (Table 2). The *cis*-oriented

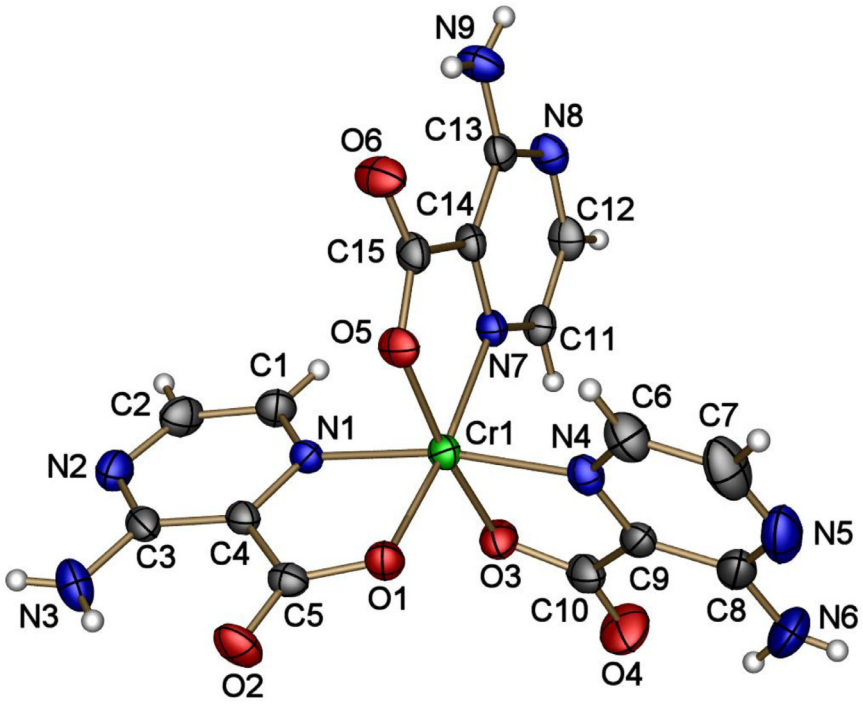


Fig. 1. The molecular structure of complex **1** with displacement ellipsoids drawn at the 50% probability level.

Table 2
Selected (a) Bond lengths (Å) and (b) angles (°) for $[(C_5H_4N_3O_2)_3Cr]$.

Cr1-O1	1.9335(19)	O1-Cr1- O3	94.35(8)
Cr1-O3	1.9378(17)	O1-Cr1- O5	91.93(8)
Cr1-O5	1.9562(17)	O3-Cr1- 05	173.14(8)
Cr1-N1	2.043(2)	O1-Cr1- N1	80.73(8)
Cr1-N4	2.057(2)	03-Cr1- N1	92.74(8)
Cr1-N7	2.058(2)	O5-Cr1- N1	90.97(8)
		O1-Cr1- N4	91.88(8)
		O3-Cr1- N4	80.78(8)
		O5-Cr1- N4	96.29(8)
		N1-Cr1- N4	169.83(9)
		O1-Cr1- N7	171.94(8)
		O3-Cr1- N7	93.35(8)
		O5-Cr1- N7	80.49(8)
		N1-Cr1- N7	96.54(8)
		N4-Cr1- N7	91.69(8)

angles are in the range 80.49(8)–96.54(8)°. The best equatorial plane is provided by the O1/O3/N7/O5 set of atoms (r.m.s. deviation = 0.0469 Å), with the metal displaced by 0.0059(5) Å toward N1. The Cr–O distances (mean value 2.04(16) Å) are not significantly shorter than the Cr–N bond distances (mean value 2.053(5) Å). The five-membered chelation rings are almost planar (maximum r.m.s. deviation = 0.0468 Å for Cr1/N1/C4/C5/O1). In general, bite distances and angles for five membered chelate rings are approximately 2.6 – 2.7 Å and 84–88°, respectively [31]. In complex **1** the O...O bite distances (2.577(3), 2.591(3) and 2.595(3) Å) and the O–Cr–N bite angles (80.74(8), 80.50(8) and 80.79(8)°) are slightly shorter/narrower because of ligand field strength and distorted octahedral environment around the metal center.

The addition on the pyrazine ring of an electron donating or electron withdrawing functional group, like -COOR, $-NH_2$, $-CONH_2$ etc., provides opportunities for more interactions suitable for the development of supramolecular architectures [32]. Due to strength and directionality of O–H…O, O–H…N and N–H…O hydrogen bonds, these interactions play a great role in the development of coordination polymers, weak interactions like C–H…O and C–H…N hydrogen bonds can also be considered as supramolecular synthons. In **1**, one amine H atom of each 3-amino-2-pyrazine carboxylato anion is engaged in an intramolecular N–H…O hydrogen bond (Table 3) forming rings of *S*(6) motif, whereas molecules are linked into 1-D *zig-zag* chain parallel to the *b* axis (Fig. 2) by the N–H…O involving the second amine H atom of only one anion (O1/O2/N1-N3/C1-C5). The chains are further connected into a three-dimensional network by C–H…O hydrogen bonds (Table 3).

3.2. Infrared spectra and X-ray powder diffraction

The vibrational spectrum of the complex is consistent with the structural data is shown in ESI 1. Peaks between 933 and 1217 cm^{-1} are assigned to the pyrazine moiety [33]. Symmetric and asymmetric N-H stretching frequencies of the amine group appear in the 3338–3456 cm^{-1} region. The broad and strong absorption band at 3338 cm^{-1} is due to the presence of O-H…X hydrogen bonding interactions. Peaks at 1317–1358 $cm^{-1}$are attributable to C-N stretch of aromatic amine [34]. The appearance of peaks at 474 cm^{-1}indicates the presence of Cr-O bond.The X-ray powder diffraction patterns of the complex are shown in ESI2. The diffraction spectra was recorded in the range of 2θ = 5–50°. The sharp peaks indicate the crystalline nature of the synthesized powder complex. The X-ray diffraction peaks of the bulk powder sample of Cr(III) and the stimulated X-ray diffraction peaks are matched exactly, by which we compare the purity of the bulk complex to the diffraction pattern obtained from single crystal X-ray diffraction pattern.

3.3. UV-Vis absorption study

The UV–vis absorption is measured with 1 × 10^{-5} mol L^{-1} methanolic solution of the ligand and the metal complex (at different concentrations) are presented in Fig. 3. From the absorption

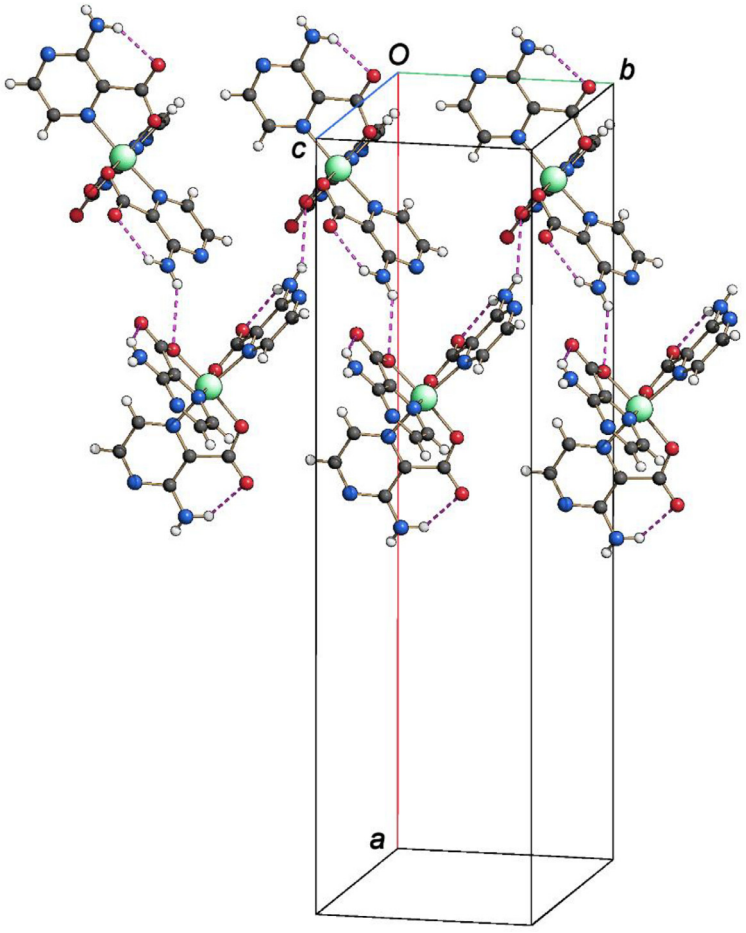


Fig. 2. Partial crystal packing of **1** showing the formation of a *zig-zag* molecular chain parallel to the *b*axis by N–H…O hydrogen bonds. Intra- and intermolecular hydrogen bonds are shown as dashed lines.

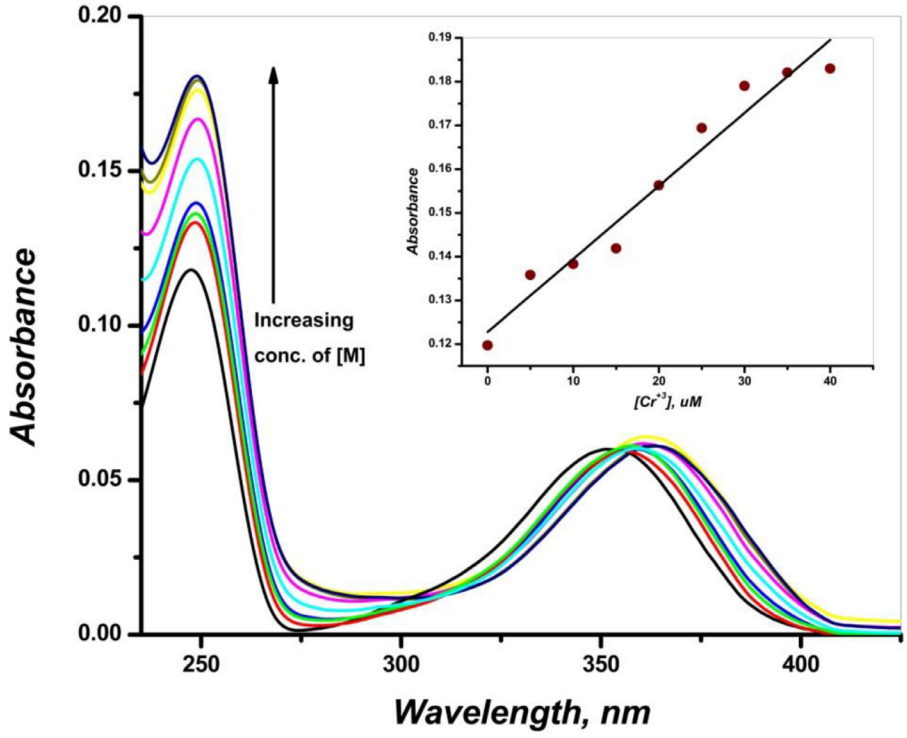
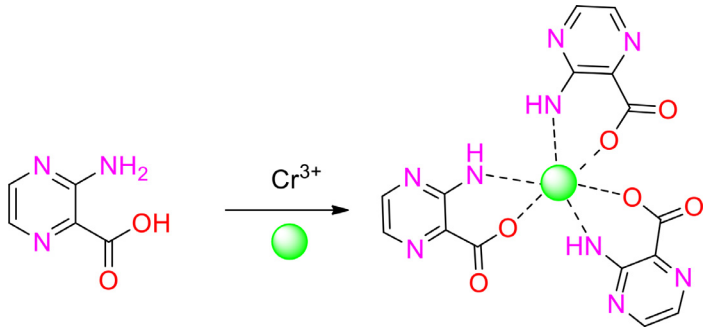


Fig. 3. Absorption spectral change of APC (1×10^{-5} mol L^{-1}) upon addition of Cr(III) in methanol. The arrows showed the increase of Cr(III)ion concentration. The concentration of Cr(III) ion was 0.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0 μM respectively. (Inset of Fig. 3: Calibration curve at 250 nm).

Table 3
Hydrogen bonds (Å and °) for $[(C_5H_4N_3O_2)_3Cr]$.

D-H···A	D-H/(Å)	H···A/(Å)	D···A/(Å)	<D-H···A/(°)	Symmetry
N3-H1N…O2	0.89(3)	2.10(3)	2.776(4)	131(3)	x, y, z
N3-H2N…O5	0.80(4)	2.48(4)	3.187(3)	148(3)	$-x + 1/2, y + 1/2, -z + 1/2$
N6-H4N…O4	0.87(4)	2.07(4)	2.737(6)	133(4)	x, y, z
N9-H6N…O6	0.84(4)	2.08(4)	2.768(3)	139(4)	x, y, z
C1-H1…O2	0.93	2.33	3.145(4)	147	$-x, 2-y, -1/2 + z$
C2-H2…O6	0.93	2.54	3.338(4)	144	x, 1 + y, z
C11- H11…O4	0.93	2.36	3.060(3)	132	-x, 2-y, -z



Scheme 1. Schematic illustration of the sensing mechanism.

maxima profile of the ligand, the higher energy band at 250 nm attributed to π-π^* transitions and lower energy band at 310 to 380 nm could be assigned to n-π^* transitions. The absorption wavelength of ligand APC (350 nm) was slightly red shifted upon addition of gradual addition of 5 μM Cr^{3+}ions. The change in absorption spectra of ligand is due to the coordination of Cr metal ions by the ligand's N and O binding sites, leads to the formation of new complex (Scheme 1). This bathochromic shift observed after addition of Cr^{3+} metal ion. Thus, the formation of a new complex leads to the relative stabilization of ground state energy alteration which correlates the bathochromic shift.

The increased in absorption intensity may be attributed to the formation of five membered chelate ring through one nitrogen and one oxygen atoms from 3-amino 2-pyrazine carboxylate moiety which enlarges the conjugated systems and reduces the energy difference between n and π^* orbital. Upon increasing the Cr^{3+} ion concentration, the band at 250 nm displays a concomitant increase in optical density. The plot of the absorbance (Fig. 3) at 250 nm generates a calibration curve [(inset of Fig. 3)] keeping the sensor concentration fixed and allows one to detect and estimate the concentration of Cr^{3+} ions present. Detection limit has calculated and the value of LOD is 0.77×10^{-6} M, by using the formula of detection limit $3\sigma/k$: Where σ is the standard deviation, k is the slope between the absorption intensity versus Cr^{3+} concentration [35]. This chemosensory response compares favourably to most of the known Cr^{3+} sensors. Comparative literature studies of chemosensors with present reported Cr^{3+} complexis given in Table S1 [21c,36–40]. From this comparison it can be inferred that our probe makes the current work more attractive and could be the simplest methodas it involves a facile one step reaction with readily available chemical.

3.4. Sensing capability in presence of interfering cations

The sensing mechanism is proposed to proceed through the metal–ligand interaction principle. Here coordination of Cr^{3+} ions with the electron rich nitrogen atom of amine moiety and oxygen atom of carboxylic acid moiety leads to the formation of five membered chelate ring formations. To examine the potentiality of this probe in presence of interfering cations we have added 10 μM Cr (III) in the mixture of other competitive cations such as Al^{3+}, Mn^{2+}, Cu^{2+}, Ni^{2+}, Fe^{3+}, Cd^{2+} and Zn^{2+}ions. Concentration of the probe was 1×10^{-5} M in methanol. From Fig. 4 (*inset*) it is observed that UV-Vis spectral response of the ligand is quite efficient to demonstrate its efficiency to distinguish trivalent chromium in the mixture of interfering cations.

Further to investigate sensor selectivity, we took different metals ions (like Al^{3+},Mn^{2+}, Cu^{2+},Ni^{2+}, Fe^{3+}, Cd^{2+},Zn^{2+}) having concentration 10 μM each in methanol towards the probe concentration 1×10^{-5} mol L^{-1} . From the responses shown in ESI 3, it is observed that the intensity of the probe slightly increases upon addition Al^{3+},Fe^{3+}, Cu^{2+}, Mn^{2+}, Cd^{2+},Ni^{2+}, Zn^{2+}.But the receptor is highly selective towards Cr^{3+}ions. Selectivity of the probe towards Cr^{3+} only, may be due to the preferential metal bonding over the other metal ionsand the generation of three dimensional *zig-zag* molecular chain parallel to the *b* axis by N–H…O hydrogen bonds (both inter and intramolecular). Probe performance towards Cr^{3+} in presence of common anions like F^-, Cl^-, NO_3^-given in ESI 4 and the results for oxoanions (like SO_4^{2-}, CrO_4^{2-}, $Cr_2O_7^{2-}$) are presented in ESI 6. The figures suggest that the probe can only effectively act as a sensor for Cr^{3+} metal ion in presence of different anions and oxo anions.From literature it is observed that compounds containing amine, thiol, hydroxyl, carboxyl groups have high affinity and coordination capability to the metal ions [41].

To study the practical applicability, the effects of pH on the probe to Cr^{3+}ions are also investigated. The experimental results (ESI 5) clearly demonstrate that the chemosensor is suitable for determining Cr^{3+}inneutral pH range (6.0–8.0), which is favorable for its application in some environmental and physiological conditions. The spectra of only ligand has been checked and provided in inset of ESI 5. The effect of neutral pH on only ligand and in presence of Cr^{3+}has also checked and given in ESI 5. Although use of methanol is restricted in some cases, the comparison of the probe with some other Cr^{3+} sensitive probes (Table S2) [35,42–45] clearly shows the superiority of this method.

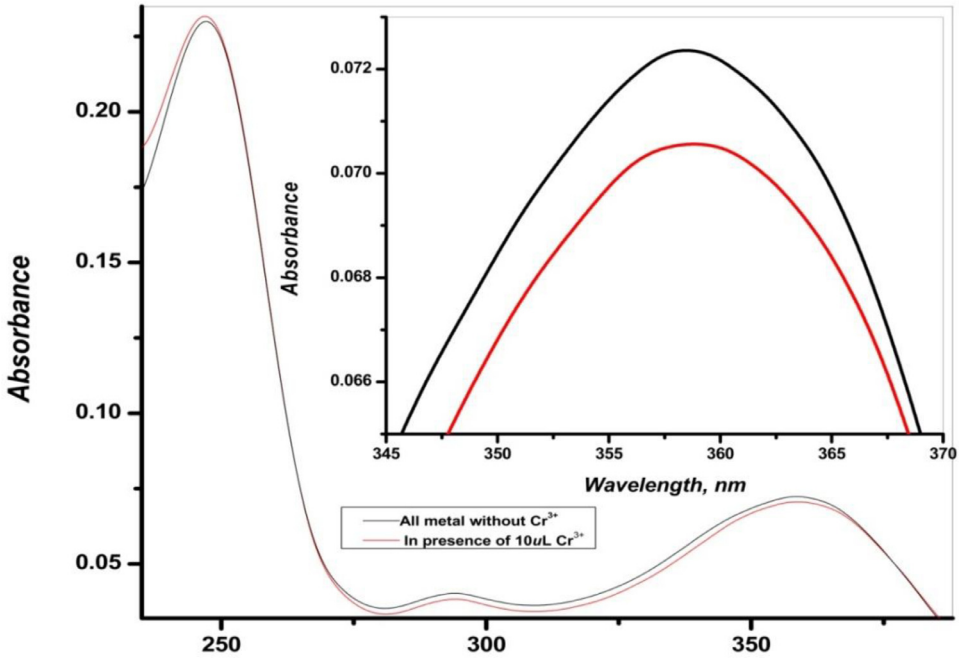


Fig. 4. Sensing capability of probe (1×10^{-5} mol L^{-1}) in the mixture of interfering cations (10 μM each) [Inset of Fig. 4. zoom view at 350 nm].

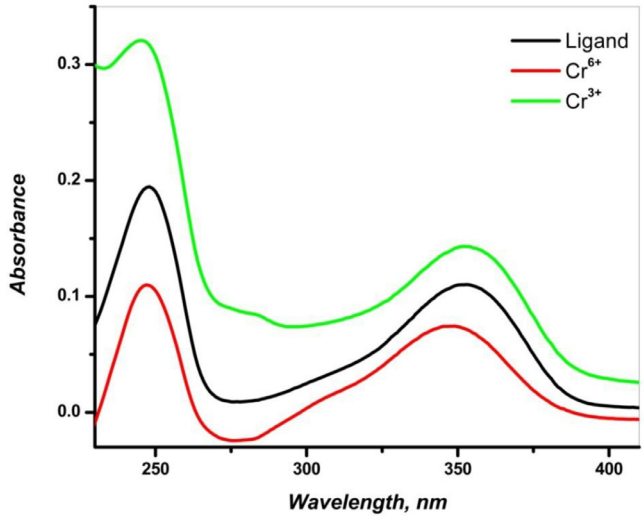


Fig. 5. Differentiation between Cr(III) and Cr(VI)with the ligand(1×10^{-5} mol L^{-1})in absorption spectrum.

3.5. Interesting opposite spectroscopic behavior

The analysis of the UV-Vis spectra also revealed an interesting feature in presence of different oxidation states of Cr in the same solvent. From Fig. 5 it is clear that the ligand shows increase in intensity for Cr(III) whereas decrease in intensity for Cr(VI) ion with respect to the probe. This type of different spectroscopic behavior for Cr(III) and Cr(VI) is due to the level of different interactions. The extent hyperchromic effect may be due to external electrostatic contacts of the formed complexes. This result clearly implies that the binding of probe with the electron rich nitrogen atom of amine moiety and oxygen atom of carboxylic acid moiety leads to the formation of supramolecular architecture, which enhanced the absorbance intensity for Cr(III) ions**.** Fate of the probe when both the Cr(III) and Cr(VI) present in a solution is given in ESI 7, indicates the presence of both but detecting specifically Cr(III) ion, based on the $\boldsymbol{\lambda_{max}}$.

3.6. Designing of molecular logic gates andsmart sensing of Cr^{+3}

The spectroscopic responses that generated from the ligand (APC) on interaction with Cr^{+3} and other metal ions (M^{+n} = Al^{3+}, Fe^{+3}, Ni^{+2}, Mn^{+2}, Cu^{+2}, Zn^{+2}, Cd^{+2}) are further exploited to design molecular logic gates. For this purpose, the ligand (APC), Cr^{+3}, and M^{+n}ions are treated as the "chemical inputs", the absorption intensity at 350 nm (I_{350}) is considered as the "optical output" and pure MeOH is considered as the initial state. A fixed threshold was considered to the 350 nm emission channel to covert the analog spectroscopic data into binary digits. The excitation intensity below and above the threshold is counted as the 'LOW' and 'HIGH' responses, respectively, and designated as binary digits '0′ and '1′, respectively. Now, considering APC and Cr^{+3} as the two chemical inputs, MeOH as initial state, and I_{350} as the single optical output, the two-input-single-output AND logic gate is designed [7].The co-presence of APC and Cr^{+3}, for the input situation (1,1), is the only case where 'HIGH' (1) output response is found at I_{350}. The other three input combinations, i.e. (0,0), (1,0), and (0,1), generate 'LOW' (0) output response. Thus, the logical response of the molecular AND gate is realized (Fig. 6a1, a2, a3). Notably, as APC can efficiently sense Cr^{+3} in solitary as well as crowded conditions, the presence of M^{+n} ions would not have any effect to the logic functioning of the molecular AND gate. Interestingly, the same molecular AND gate can be modulated to perform the logic action of two-input-single-output PASS 0 gate through simple alteration of the 'chemical input' Cr^{+3} to M^{+n}, considering all the other chemical and optical parameters intact. Thus, considering APC and M^{+n} as the two chemical inputs, MeOH as preliminary state and I_{350} as optical output, the PASS 0 gate is realized (Fig. 6b1,b2) [46].The absorption intensity at 350 nm for all the four input situations, (0,0), (1,0), (0,1), and (1,1), are found to be well below to the applied threshold, ultimately generating 'LOW' (0) output response. Thus, in absence of Cr^{+3}, we would observe the PASS 0 logic response. Hence, we would expect the logic responses of the AND or PASS 0 gates based on the presence or absence of Cr^{+3}, respectively. Ulti-

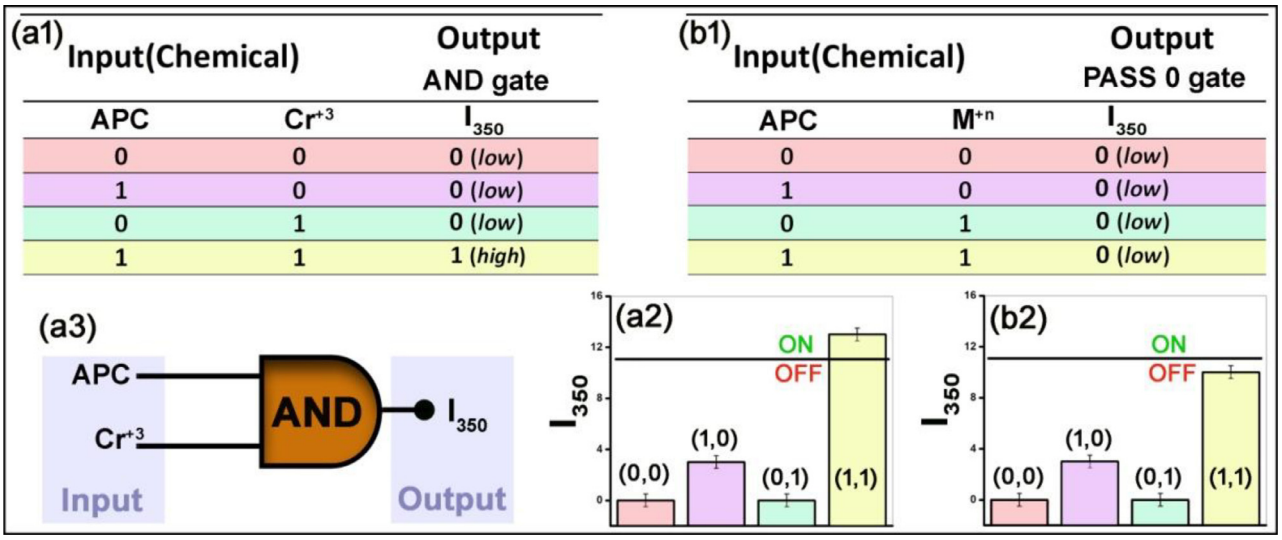


Fig. 6. (a1) Truth table, (a2) bar diagram and (a3) logic gate representation of molecular AND gate; (b1) Truth table and (b2) bar diagram of molecular PASS 0 gate.

mately, all these observations could be taken into account for the smart-sensing of Cr^{+3} via differential logic actions by utilizing the ligand, APC.

4. Conclusion

Chemosensing property of 3-amino-2-pyrazine carboxylic acid (APC) is explored by using UV-Vis spectroscopy, and employed as a selective optical chemo sensor for chromium (III) ions. The binding of the probe with Cr(III)is firmly established by the SCXRD technique that was isolated from APC under solvo-thermal conditions. Structural analysis revealed that the mononuclear Cr(III) complex forms 3-D network by C–H…O hydrogen bonds. Selective sensor property was observed for Cr(III) in presence of interfering ions. The experiments were repeated for three times keeping the concentration same. Another interesting property is that it exhibits opposite spectroscopic behavior for two different oxidation states of Cr. Thus the ligand can differentiate Cr(III) and Cr(VI) with the help of a simple spectrophotometric technique. The developed method is very simple, offers high sensitivity and has the advantage of low cost and does not need any extra fluorophores for the sensitive detection of both the forms of chromium. The spectroscopic responses generated from the ligand (APC)on interaction with Cr^{+3} and other metal ions (M^{+n} = Al^{3+}, Fe^{+3}, Ni^{+2}, Mn^{+2}, Cu^{+2}, Zn^{+2}, Cd^{+2}) are used to design molecular logic gates.

Authorship statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the Journal of Molecular Science.

Supplementary materials

For the structural analysis crystallographic data have been deposited with the Cambridge Crystallographic data center, CCDC No. 2108329. Copy of this information may be obtained free of charge from The Director, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Abhishikta Chatterjee: Funding acquisition, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Priyanka Chakraborty:** Data curation, Formal analysis. **Bidyapati Kumar:** Data curation, Formal analysis. **Corrado Rizzoli:** Funding acquisition, Data curation, Formal analysis. **Pinaki Mandal:** Writing – review & editing. **Subrata K. Dey:** Conceptualization, Visualization, Writing – original draft, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2022.132486.

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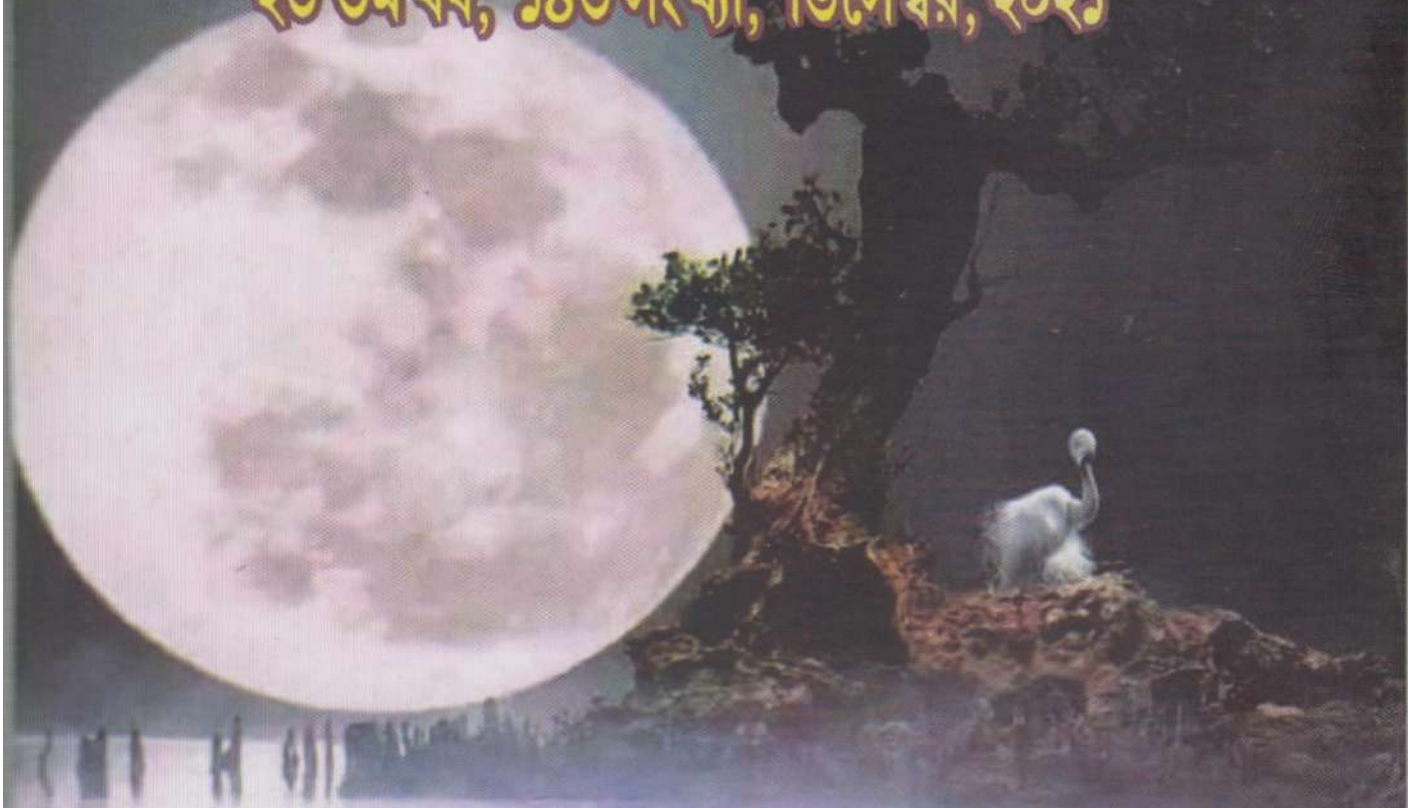
'এবং মহুয়া' –বিশ্ববিদ্যালয় মঞ্জুরী আয়োগ (UGC-CARE list-I 2021) অনুমোদিত তালিকার অন্তর্ভুক্ত। ২০২১সালে প্রকাশিত ১৬পৃ.তালিকার (৩১৯টির মধ্যে) ৩ পৃ.৬০নং উল্লেখিত।

এবং মহুয়া

(বাংলা ভাষা, সাহিত্য ও গবেষণাধর্মী মাসিক পত্রিকা)

২৩ তম বর্ষ, ১৪৩ সংখ্যা, ডিসেম্বর, ২০২১



সম্পাদক
ডা. মদনমোহন বেরা

কে.কে. প্রকাশন
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বিশ্ববরেণ্য ছৌ-শিল্পী গম্ভীর সিংমুড়া : জীবন ও কাহিনি

ড. সমরকান্তি চক্রবর্তী

ভূমিকা :

'ছৌ' মুখাবরণ পরিহিত নৃত্য। মুখকে কেন্দ্র করেই মুখোশের আবির্ভাব। পৃথিবীতে আদিম জনগোষ্ঠী কর্তৃক যে সভ্যতাগুলির উদ্ভব ও বিকাশ ঘটেছিল, সেই সভ্যতার শিরায়-উপশিরায় মুখোশ নৃত্যের বিভিন্ন স্বরূপ আজও পরিলক্ষিত হয়। পশ্চিমবাংলার পশ্চিমতম প্রান্তের জঙ্গল মহলের অন্তর্গত জেলাগুলিতে, বিশেষ করে পুরুলিয়ায় ছৌ-নৃত্য অত্যন্ত জনপ্রিয়। অহল্যাভূমি পুরুলিয়ার রুক্ষমাটি, ডুংরি পাহাড়ের অপরূপ নন্দনকানন, জোর-বাঁধের স্বচ্ছ জলরাশি, নদ-নদী, বনজ ও খনিজ সম্পদে যেমন সমৃদ্ধ, তেমনি এখানে রয়েছে লোক-সংস্কৃতির বিপুল ভান্ডার। ছো থেকে আরম্ভ করে টুসু, ভাদু, বাদনা, করম, পাতানাচ, নাচনিনাচ, বিহাগীত, প্রভৃতি কি নেই এখানে? এখানকার আদিবাসী নৃত্য ও লোককাহিনি অবহেলিত, উপেক্ষিত জেলাটির মান-মর্যাদা অনেকগুণ বাড়িয়ে দিয়েছে। লোকসংস্কৃতির গবেষকদের মতে প্রতিটি উৎসব বা পার্বণের নেপথ্যে একটি প্রেক্ষিত থাকে এবং তা গড়ে ওঠে সেখানকার ভূপ্রকৃতি, খাদ্যভাস, ভাষা, দৈনন্দিন জীবনযাত্রা, পারিপার্শ্বিক পরিবেশের উপর ভিত্তি করে। এই প্রবন্ধে মূলত লোক-নৃত্যের অন্যতম প্রবাদ প্রতিম নৃত্য-শিল্পীর অজানা জীবন সংগ্রাম ও কর্ম-যজ্ঞের নানা কাহিনির সম্পর্কে সংক্ষিপ্ত আলোকপাত করার চেষ্টা করছি। এই বিশ্ববরেণ্য নৃত্য-শিল্পীর কথা ও কাহিনি আলোচনার পূর্বে মানভূমের ছৌ-নৃত্য কী? এর উদ্ভব, বিকাশ ও বৈশিষ্ট্য সম্পর্কে সংক্ষিপ্ত ধারণা থাকা অত্যন্ত আবশ্যক। নাহলে আন্তজাতিক শিল্পীর গুরুত্ব অনুধাবন করা অসম্ভব বলেই মনে হয়।

ছৌ বলতে কি বোঝায়?

'ছ' বা 'ছউ', কিংবা 'ছো' নাকি 'ছৌ' – কোনটা? বিতর্কিত এই শব্দটি ঘিরে নানা মুনির নানা মত। বিগত তিন দশকেও এর সমাধানের জট খোলেনি। আশুতোষ ভট্টাচার্য এবং তাঁর গবেষক ছাত্র-ছাত্রীরা 'ছৌ' শব্দটিকে প্রাধান্য দিলেও পশুপতি মাহাত, বিভূতিভূষণ দাশগুপ্ত, ডঃ বঙ্কিম মাইতি, ডঃ শান্তি সিংহ প্রমুখেরা 'ছৌ' না বলে 'ছো' বলার পক্ষপাতি। ঝুমুর শিল্পী সলাবত মাহাত ঝুমুরেও ছো কথার উল্লেখ রয়েছে। অর্থাৎ দেশীয় ছৌ ও ঝুমুর শিল্পে প্রবাদ-প্রতিম ব্যক্তিত্বগণের মধ্যে 'ছো' শব্দবন্ধ ব্যবহার করার রীতি বা ধরণ প্রচলিত আছে।[১]

"পুরুলিয়ার ছোনৃত্য সারা বিশ্বে বিখ্যাত
অমর হয়ে রহিল ধরায়"।

নৃত্যের তালে ও ছন্দে ছয় জন শিল্পীর নিখুঁত যুগলবন্দীর কথাও উড়িয়ে দেওয়া যায় না। আবার কুড়মালি ভাষা ও সংস্কৃতি বিশেষজ্ঞ প্রদীপ কুমার মাইতির, কোনো দক্ষ ব্যক্তির বিশেষ কোনো কাজে তাঁর প্রতিভাকে সুস্পষ্ট ভাবে প্রতিভাত করার কৌশলকে এবং বিশেষ ভাষাকে 'ছউ' বা 'ছ' বলে অভিহিত করেছেন। আবার তিনি এও বলেছেন এই নাচে যে মুখোশ ব্যবহৃত হয় তাকে কুড়মালিতে 'মহ্রা' বলে। মহ্রা কথাটি তরজমা করলে দাঁড়ায় এইরূপ – মুহ (মুখ)+উঢ়া /অঢ়া (ঢাকা) = 'মহ্রা' (মুখ ঢাকা)।[২] এই 'মহ্রা' শব্দটির আদি পরবর্তীকালে মুখোশ শব্দটি এসেছে। তাই ছোটনাগপুর অঞ্চলে মহ্রা শব্দটির চল রয়েছে।

কোনো সৃষ্টিই আপনা আপনি হয় না, এর পিছনে থাকে চাহিদা, তাগিদ, নিষ্ঠা, পরিশ্রম এবং জীবনের ছন্দ। মাটি হল সৃষ্টির ভিত্তি ভূমি। আর সৃষ্টির সঙ্গে কৃষ্টি যুক্ত হয়ে জন্ম নেয় সংস্কৃতি।

বর্তমান প্রতিমুহূর্তে অতীত কে পিছনে ফেলে ভবিষ্যতের দিকে অগ্রসর হয়ে চলেছে। এভাবেই আসে পরিবর্তন। আবার এক সময় পরিবর্তনেরও পরিবর্তন ঘটে। রচিত হয় ইতিহাস।মানভূমের প্রায় অখ্যাত ছৌ নৃত্যকে অকস্মাৎ যেন বিশ্বদরবারে সম্মানিত করল চড়িদা গ্রামের বাবু সিং। বর্তমানে ছৌ নৃত্যের আঁতুড় ঘর যে পুরুলিয়ার রাঙামাটি, লালমাটি – তা বিদ্দজনেরা একবাক্যে স্বীকার করেন। তবে পুরুলিয়ার ছৌ নৃত্যে মুখোশের প্রাধান্যই অগ্রগণ্য।

বিশেষ করে একটি বিশেষ সম্প্রদায়ভুক্ত জনগোষ্ঠী এই শিল্পের সঙ্গে যুক্ত। তারা হলেন– হো, সাঁওতাল, কর্মী, ওরাং, মুন্ডা, মালপাহাড়ি ভূমিজ প্রভৃতি প্রাচীন উপজাতি শ্রেণির মানুষ। ডঃ বঙ্কিম মাইতির মতানুসারে ছৌ-নাচে প্রধানত কুর্মি-মাহাত এবং ভূমিজ সম্প্রদায়ের মানুষই অংশ গ্রহণ করে থাকেন।

গম্ভীর সিং মুড়া :

ছৌ নারী বর্জিত নৃত্য। পৌরষ-দৃপ্ত পুরুষ শিল্পীরাই নারী চরিত্রে অভিনয় করে থাকেন। তবে বর্তমানে নারীরা এই নৃত্যে নৃত্য প্রদর্শন করে তার আকর্ষণকে আরও বৃদ্ধি করেছে। শারীরিক কলা কৌশলের মাধ্যমে কিছু শিল্পী কৃতিত্বের অধিকারী হয়েছেন। এদের মধ্যে এমন একজন নজর কাড়া শিল্পীর কথা আলোচনা করব যিনি পুরুলিয়া জেলার পরিচিত বিশ্বের দরবারে পৌঁছে দিয়েছেন। কৌতূহলী মনে প্রশ্ন জাগবেই তিনি কে?

সেই প্রসঙ্গে আসছি। তাঁর কথায় আসছি। আপনারা কি কখনো অযোধ্যা পাহাড়ের নাম শুনেছেন? বাগমুন্ডি থানা এই স্থানটি পর্যটক অর্থাৎ প্রকৃতি প্রেমীদের কাছে একটি অতি পরিচিত নাম। ডুংরি বা টুংরি পাহাড়ের সারি, ড্যাম, টানেল,

ঝরণা, চা বাগান পাশেই পাখির পাহাড় প্রভৃতি প্রাকৃতিক সৌন্দর্যের মোড়াস্থানটি দেশ-বিদেশের পর্যটকদের নজর কাড়ে। ইচ্ছে হলে আপনিও ...।

আর এই পাহাড়ের কোলে শাল, পিয়াল, পলাশ, কুসুম, শিমুল, সেগুন প্রভৃতি জঙ্গল ঘেরা ছোটগ্রাম-চড়িদা। অনেকটা দক্ষ শিল্পীর আঁকা ল্যান্ডস্কেপ বা নিসর্গদৃশ্যের মতো।

এই গ্রামে বাস করেন কৃষ্ণ বর্ণের, শীর্ণকায়, স্বল্প বাক্, নিরক্ষর মানুষটি। তিনি মোটেই গম্ভীর নন বরং শান্ত প্রকৃতির। আর তাঁর মাথায় কোনো সিং নেই। রয়েছে একরাশ ঝাঁকড়া লম্বা চুল এবং বিশাল গোঁফ।[৩] চোখে দূরদৃষ্টি, পেটে সর্বক্ষণ ক্ষুধা। পোশাক-পরিচ্ছদে বাহুল্য নেই। বেশির ভাগ সময়ই লুঙ্গি-গেঞ্জি, কখনো উদোম গায়ে হাঁটু পর্যন্ত মালকোঁচা মারা ধুতি পরিহিত। বিড়ি ফুঁকতেন আর দেশলাই জেলে মাঝে মধ্যে গলা ভেজাতেন। হাতে একটি বালা পড়তেন। পদ যুগল অধিকাংশ সময় মাটির সঙ্গে মিশে থাকত। শিক্ষাদিক্ষা বা পড়াশোনার কথা আর কি বলব! প্রাথমিকের চৌকাট পর্যন্ত পেরোতে পারেননি। দারিদ্রতার উত্তাল সমুদ্র তাকে বারবার বিভ্রান্ত করেছে। তাই সে, কোনো মতে টে নে টুনে ক্লাস টু বা দ্বিতীয় শ্রেণি উত্তীর্ণ হয়েছেন। কিন্তু না, একদম তাচ্ছিল্য বা অবজ্ঞা করবেন না। কারণ তার যা আছে আমাদের অনেকেরই তা নেই। বরাই করে গুটি কয়েক বই পড়ে ডিগ্রী লাভ করা যায়। কিন্তু তা দিয়ে কখন প্রতিভার বিচারকরা সম্ভব নয়। প্রতিভা অসীম এবং দুর্লভ। তাই সেই দ্বিতীয় শ্রেণির উত্তীর্ন ছাত্রটি প্রতিভার জোরে সাফল্যের শীর্ষ চূড়াকে স্পর্শ করেছেন। তিনি আর কেউ নন তিনি হলেন আন্তজাতিক খ্যাতি সম্পন্ন প্রবাদ-প্রতিম শিল্পী পদ্মশ্রী গম্ভীর সিংমুড়া ওরফে বাবু সিং।

ছোটনাগপুর মালভূমির এই অন্ত্যজ শ্রেণির ভূমি পুত্রের জন্ম হয় ১৩৩২ সালের ১৮ইফাল্গুন (ইং—১৯৩০ সালে) মাতুলালয় পিটদিরি গ্রামে। পিতা জিপা সিং মাতা ফুলমনি মুড়া।[৪] প্রকৃতির কোলে ক্ষুদ্র কুটিরে আটপৌরে জীবনযাত্রা। কোন কারণে রাজা মদন মোহনের রোষানলে পড়ে দরবার থেকে বিতারিত হন জিপা। জীবনে বহু উত্থান পতনের পর চরিদায় আগমন। মাত্র দুবছর বয়সে বাবু-বাবাকে হারান। প্রতিভা ছিল, তাই বাবার সুযোগ্য শিষ্য মধুভাট রায়ের কাছে তালিম নেন বা 'ছো' শিক্ষার হাতে খড়ি সমাপ্ত করেন। বাঘমুন্ডির রাজা ক্ষেত্র মোহনের শাগরেদ গোলাম মহম্মদ খানের কাছে জীপা তনয়ের প্রতিভার কথা শোনেন। চৈত্র সংক্রান্তির গাজনে রাজদরবারে গম্ভীরের ডাক পড়ে। শোনা যায় সেখানে একলপ্তে ১০০টি ডিগবাজি খেয়ে সকলকে তাক লাগিয়ে দিয়েছিলেন। মুগ্ধ রাজা, মুগ্ধ দর্শকবৃন্দ মন্ডলী। রাজা তাঁর নৃত্যে মুগ্ধ হয়ে তাকে তিন কুঁড়ি টাকা এবং এক হাঁড়ি রসগোল্লা উপহার দেন।[৫]

বাল্যকালে গোচরণে যেতেন গম্ভীর। একদিন লক্ষ করেন একটি ফুটফুটে বকনা বাছুর ছানা মনের আনন্দে এদিক ওদিক লাফিয়ে বেড়াচ্ছে। এই দৃশ্যটি তাঁর মনের গভীরে দাগ কাটে, প্রেরণা জন্মায়।[৬] ক্ষেত্র সমীক্ষায় একান্ত সাক্ষাৎকারে লেখক

স্বপন কর্মকারকে শিল্পী স্বয়ং এই কথা বলেছিলেন। সম্ভবত আজ পর্যন্ত কোথাও একথা প্রকাশিত হয়নি।

তিনি শারীরিক কসরতের মাধ্যমে ঐ অঙ্গিভঙ্গি ও মোচরগুলি ভালোভাবেও অনুশীলন করেন। যথার্থ ভাবে আয়ত্ত করেন। কখনো একা, আবার কখনোবা দল বেঁধে, নদীর বালুচরে, পুকুরে বা জলাশয়ের উন্মুক্ত নন্দনকাননে, কখনো ময়দানে বা খড়ের গাদায় হাঁটুপুটকা, উল্কা (শূন্যে গা ভাসিয়ে দেওয়া), সামনে এবং পেছনে গাড়ির চাকার মতো প্রভৃতি কৌশলগুলি নিরলস নিয়মানুবর্তিতার মাধ্যমে আয়ত্ব করেছিলেন। তাঁর ছৌ শিক্ষা প্রকৃতির পাঠশালায় শুরু হয়েছিল, তাই তাঁর নৃত্যের প্রকৃতির অলৌকিক শক্তির প্রতিফলন বিশেষ ভাবে লক্ষণীয়। জীবনের শেষ দিন পর্যন্ত এই বিদ্যাকে সুকৌশলে ব্যবহার করেছেন। কখনো মহিষাসুর, কখনো অভিমুন্য, কখনো কিরাত প্রভৃতি নানা চরিত্রে তাঁর মন মুগ্ধকর নৃত্যাভিনয় ও ছন্দবদ্ধ শরীরের অভূতপূর্ব কৌশল বাংলা তথা পৃথিবীর মানুষকে আনন্দদান করেছিল। তিনি দর্শকদের মুগ্ধ, মনোরঞ্জন করতেন। অসাধারণ নৃত্য প্রদর্শনের জন্য দেশ-বিদেশ থেকে প্রচুর ডাক আসতো। স্বদেশের মাটিতে তিনি যেমন দাপিয়েছেন তেমনি তিনি অর্ধেক পৃথিবী নৃত্যের কর্ষণে কর্ষিত করেছেন। আর সম্মান বলতে পদ্মশ্রী পুরস্কারের কথা বলছি। বিদেশে অর্থাৎ ইউরোপ, উত্তর আমেরিকা, প্যারিস, জাপান প্রভৃতি দেশে ও মহাদেশে পুরুলিয়ার ছৌ-নৃত্যকে জীবনের শেষ রক্তবিন্দু দিয়ে সম্মানিত করেছেন।[৭] তাকে নিয়ে নানা তথ্যচিত্র, ডকুমেন্টরি ইত্যাদি হয়েছে।

দেশ-বিদেশে নৃত্য প্রদর্শন ও স্বীকৃতি লাভ :

পদ্মশ্রী পুরস্কার- ১৯৮১ সালে ১৮শে মার্চ (রাষ্ট্রপতি নিলাম সঞ্জীব রেড্ডি)।

অ্যাকাদেমি পুরস্কার- ১৯৮২ সালে ২১শে মার্চ (রাষ্ট্রপতি জ্ঞানি জইল সিং)।

চিত্র গ্রহণ : পুরুলিয়াতে ঋতিক ঘটক (তাং.....)

মৃনাল সেন ছবি করেছেন মৃগয়াতে। দুমকা জেলার ময়ুরাক্ষী নদীর তীরে।[৮]

নৃত্য প্রদর্শন :

১৯৭২ সালে (ইউরোপে)- লন্ডন, প্যারিস, হল্যান্ড, স্পেন প্রভৃতি (৪৫দিন)

১৯৭৫ সালে (উত্তর আমেরিকা) – নিউইয়র্ক, সানফ্রানসিসকো, ওয়াটার লু, ক্যানাবিন, লস অ্যাঞ্জেলস (হাওয়াই) হিলো-হেনলালো (২ মাস)

১৯৮৬ সালে – ইন্ডিয়ান ফ্যাস্টিভাল অফ প্যারিস- বিভিন্ন শহরে ৪৫ দিন।

১৯৯১ সালে - ইন্ডিয়ান ফ্যাস্টিভাল অফ জাপান – (ওসাকা) ১ মাস। ইত্যাদি।[৯]

এই মুখোশ নৃত্য 'ছৌ নৃত্যভূমি পুরুলিয়া'র ছৌ সংস্কৃতির মর্যাদার রাজমুকুটে একনতুন পালক সংযোজন করেছে। এই জেলার বিশ্ববরেণ্য ছৌশিল্পী গম্ভীর সিংমুড়ার অভূতপূর্ব ছৌ-নৃত্য প্রদর্শনের মাধ্যমেই বঙ্গ সংস্কৃতির প্রাচীন নৃত্য, মুখোশ নৃত্য, শতশত বিশ্ববাসীর ভালোবাসা ও প্রশংসায় সম্মানিত হয়েছে। ছৌনৃত্যে তাঁর নব

ভাবনার মাধুরীর সঞ্জীবন ঘটেছিল। ১৯৭২ সাল থেকে ইউরোপ, এশিয়া ও আমেরিকার নানা প্রান্তে অসীম ছন্দে তাঁর অপরূপ নৃত্য প্রদর্শন, ঘুঙুরের ঝংকার, ঢামসা মাদলের দিদির দাং, দিদির দাং, দিদির দাং শব্দের মাদকতা যেন সভ্য দেশের অগণিত শ্রোতা ও জ্ঞানী-গুনী মানুষকে মুগ্ধ করেছিল।[১০]

অখ্যাত-অজ্ঞাত বাঘমুন্ডির চড়িদা গ্রামের পদ্মশ্রী পুরস্কার প্রাপক গম্ভীর সিং-এর জীবন কাহিনির অন্তরালে এক অবিশ্বাস্য সংগ্রামের কাহিনি নিহিত আছে। ছোটবেলা থেকেই মুখে অনাবিল আনন্দ থাকলেও আর্থিক দৈন্যদশার কষাঘাতের স্রোতে তাঁর হৃদয়কে, তাঁর স্বপ্নকে বার বার এক অনিশ্চিত ভবিষ্যত ও সংশয়ের সম্মুখে দাঁড় করিয়েছিল। এই অব্যক্ত যন্ত্রনার মধ্যেই পুরুলিয়া জেলার আড়ষা-বাঘমুন্ডি ইত্যাদি অঞ্চলের পর পর প্রাকৃতিক দুর্যোগ ও দুর্ভিক্ষ তাদের হত দরিদ্র পরিবারকে নিদারুন ভাবে আঘাত করেছিল। ১৯৫৩, ১৯৬১-৬২, ১৯৭১-৭২, ১৯৮১-৮২ সালের মমান্তিক খাদ্য সংকট, রাজনৈতিক দোদুল্যমান অবস্থা ইত্যাদি তাঁর স্বপ্নকে বিলম্বিত করতে না পারলেও তাঁর সহধর্মিণী ও পুত্র সন্তানের উপর নির্মম আঘাত হেনেছিল।

উপরিউক্ত সালের দুর্ভিক্ষের কথা সরকারি দলিল দস্তাবেজে পরিষ্কারভাবে উল্লেখ আছে। এই দুর্ভিক্ষের চোরা স্রোতে তাঁকে পরিযায়ী শ্রমিকে পরিণত করতে পারত। পরিবারের দুঃখ দুর্দশার করাল গ্রাস তাঁর স্বপ্ন ও চরিত্রবলকে ভেঙ্গে চুরমার করে দিতে পারত। গ্রীষ্মের প্রচন্ড দাবদাহ তাদের জীর্ণ কুটিরকে যেমন উত্তপ্ত করত, তেমনি প্রায় প্রতি বছর কালবৈশাখী ঝড়ের তান্ডব তাদের ঘরকে তছনছ করে দিত। এখানেই কি দুর্দশার শেষ? না! প্রচন্ড বৃষ্টি তাঁর জীর্ণ কুটিরে সাময়িক প্লাবন করত। এই দুঃসময়ে গম্ভীর সিং চোখের জলকে শাসন করে সকল পরিবারের সদস্যকে সান্ত্বনা দিতেন। ক্রমাগত দারিদ্রের অসহ্য যন্ত্রণায় তাঁর সহধর্মিণীর চোখের জল শুকিয়ে গিয়েছিল। তথাপি মধ্যযুগের অসীম শক্তি ও সাহসী চরিত্রের অধিকারী চাঁদ সওদাগরের ন্যায় তাঁর নিজ প্রতীক্ষায় অবিচল ছিলেন। তাঁর সেই স্বপ্ন হল অ-নামী, অখ্যাত বাঘমুন্ডির ছৌ নৃত্যকে দেশ তথা আন্তজাতিক মঞ্চে সম্মানিত করা। এই সম্মান সহজে আসেনি। সহধর্মিণীর শারীরিক ব্যাধি উপেক্ষা করে, পিতা-পুত্রের স্নেহের বন্ধনকে জয় করে, নিঃস্ব হস্তে সংযোগকারীর উপর গভীর বিশ্বাস রেখে ১৯৭৫ সালে পৃথিবীর সবথেকে উন্নত ও শক্তিশালী দেশ উত্তর আমেরিকার নিউইয়র্কে, সানফ্রান্সিসকোতে, ওয়াটারলু, লসঅ্যাঞ্জেলেসে ছৌ নৃত্য প্রদর্শন করে আমেরিকা তথা পাশ্চাত্য দেশের দৃষ্টি আকর্ষণ করেছিলেন। পদ্মশ্রী পুরস্কার তাঁর প্রায় অনেক পরে লাভ করেন। কিন্তু ততদিনে তাঁর পুত্র সন্তানের স্বপ্নের দিনগুলি দারিদ্রতায় শেষ হয়ে গেছে। তারা শিক্ষা থেকে বঞ্চিত, বঞ্চিত অন্ন, বস্ত্র, বাসস্থান থেকে। গম্ভীর সিং যেন সন্ন্যাসীর মতো ত্যাগী। তিনি নিজের জীবনকে উৎসর্গ করেছেন দেশ ও দশের জন্যে। আর তাঁর সহধর্মিণী চিন্তামণি সমাজ সংস্কারের সুখ-শান্তি হতে এক বঞ্চিতা নারী। এখানে সংগ্রাম দুই ধরণের। একটি হল গম্ভীরের স্বপ্ন ও ছৌ-নৃত্যের আন্তজাতিক খ্যাতি বৃদ্ধি। সেখানে

তাঁর নিরলস নিয়মানুবর্তিতা, চর্চা ও অগ্নিপরীক্ষার প্রমাণ তাঁকে বার বার দিতে হয়েছে। অন্যদিকে তাঁর পরিবারের আর্থিক সংকট, দৈন্যদশা, খাদ্যাভাব, সাহায্যের অভাব ইত্যাদিকে জয় করে চিন্তামণি দেবী লোক চক্ষুর অন্তরালে বাঘমুন্ডির ছৌ-নৃত্যের প্রচার প্রসারে যে সংগ্রামী জীবন অতিবাহিত করেছিলেন তা স্বাধীনোত্তরকালে অত্যন্ত বিরল। অত্যন্ত শীর্ণকায়া হলেও মনে অদম্য জেদ যেন ঔপনিবেশিককালে বিপ্লবী সশস্ত্র নারীদের কথা স্মরণ করিয়ে দেয়। এইভাবে গম্ভীরসিং ও চিন্তামণির সংগ্রামের ক্ষেত্র ছিল পৃথক, কিন্তু দুজনেই সংগ্রামী। তাঁর দীর্ঘ জীবন সংগ্রামের সূর্যোদয় ক্রমশই জীবন সূর্যাস্তের কোলে ঢলে পড়েছিল। ইতিমধ্যেই তাঁর সহধর্মিণী কয়েকবার মৃত্যুর হাত থেকে বেঁচে গেলেও যমরাজের নিদারুণ আঘাত তাঁর চোখে মুখে শরীরে যেন স্পষ্ট।

গম্ভীর সিংও যেন বয়সের ভারে অনেকটাই ন্যুব্জ। তাঁর দীর্ঘ ৭৭ বছরের জীবন সংগ্রামের রক্তঝরা পারফর্মেন্সের (Performance in Chhow Dance) যেন ইতিহাসে ডুব দিয়েছে। যে গম্ভীর সিং একদিন শরীরের নান্দনিক যাদুতে ও পদযুগলের ছন্দময় আস্ফালনে লক্ষ লক্ষ ভক্তবৃন্দকে মুগ্ধ করত, বিশ্বের নানা মঞ্চে দাপিয়ে বেড়াত, আজ সে নানারকম দুরারোগ্য ব্যাধিতে আক্রান্ত। হয়তো তাঁর ছৌ-নৃত্যের জাদুতে অনেক অর্থলাভ হয়েছিল কিন্তু তা দিয়ে গম্ভীর সিং সেরকম গৃহ, সম্পত্তি কিছুই করতে পারেনি? নব্বইয়ের দশকে তাঁর জরাজীর্ণ কুটির যেন নিযাতিত ও নিষ্পেশিত যুগের কথা স্মরণ করিয়ে দেয়। তিনি মানুষের মণিকোঠায় সম্মানিত হলেও সরকারি সুযোগ সুবিধার সাহায্য থেকে ছিলেন বঞ্চিত। এইভাবে আর্থিক দৈন্যদশায় ক্রমান্বিত আঘাত ও দীর্ঘ পরিশ্রমের ফলে তিনি এবং স্ত্রী প্রায় মৃত্যুর কোলে ঢলে পড়ে। তারা যেন রাষ্ট্র উদাসীনতার সাক্ষী।

এইভাবে এক চাপা অভিমান নিয়ে ছৌ-নৃত্যের যাদুকর, গম্ভীর সিংহের যে পদ যুগলের আস্ফালনে দর্শক মুগ্ধ হতেন, মাতাতেন। নিয়তির অমোঘ নিয়মে, রবিবার স্তব্ধ হয়ে গেল (ইংরেজি ২০০২ সালে ১০ই নভেম্বর, বাংলা ২৩শে কার্তিক ১৪০৯) তাঁর জীবন সংগ্রাম।[১১]

কলকাতা থেকে ডাক্তার দেখিয়ে ফেরার পথে ট্রেনের মধ্যে আকস্মিক তাঁর মৃত্যু হয়, ছায়া সঙ্গী ছিলেন স্ত্রী চিন্তামনি। রেখে গেলেন প্রজন্ম ও উত্তরাধিকারীদের (৩ পুত্র সন্তান- কার্তিক, গণেশ ও পরশুরাম)। তার নাগরিকত্ব নিয়েও প্রশ্ন উঠেছিল। ১৯৫১ সালে স্বপন কুমার কর্মকার তাঁর এক প্রতিবেদনে জানিয়েছিলেন যে, নুন আনতে পানতা ফোরানোর দৈন্যদশা কাটিয়ে উঠলেও মহান শিল্পীর শ্রীহীন খসে পড়া খোলা বাড়িতে এখনো দারিদ্রের ছাপ স্পষ্ট।[১২] পিছনে আদিবাসী ছৌ নৃত্য দলের পার্টি অফিসেরও একই হাল। দারিদ্রতা যতই থাকুক না কেন, প্রতিভা ও সত্যের কাছে সবই ম্লান হয়ে যায়। গম্ভীর সিং মুড়া তারই জ্বলন্ত উদাহরণ। তাঁর চরিত্রবল ছিল, তাঁর বড় সম্পদ। তাই ইতিহাসের নিয়মেই তিনি নিজেকে বিশ্ব-পাদ প্রদীপের সামনে উপস্থাপন করেছিলেন। বর্তমানে চোখে পড়বে অযত্নে দন্ডায়মান গম্ভীর সিং মুড়ার মর্মর

মুর্তিটি! যদিও ফলকের লেখাটি জ্বলজ্বল করছে। পরিশেষে এই কথা বলা যায় যে, দারিদ্রতা যতই থাকুক না কেন, প্রতিভা ও সত্যের কাছে সবই ম্লান হয়ে যায়। গম্ভীর সিং মুড়া তারই জ্বলন্ত উদাহরণ। তাঁর চরিত্রবল ছিল, তাঁর বড় সম্পদ। তাই ইতিহাসের নিয়মেই তিনি নিজেকে বিশ্ব-পাদ প্রদীপের সামনে উপস্থাপন করেছিলেন। পুরুলিয়া জেলায় যতদিন ছৌ নৃত্য থাকবে লোক সংস্কৃতির ইতিহাসের পাতায় বিশ্ববরেণ্য গভীর সিং মুড়ার নাম ততদিন স্বর্ণাক্ষরে লেখা থাকবে।

তথ্যসূত্র :

১. আশুতোশ ভট্টাচার্য, 'বাংলার লোক সংস্কৃতি', ন্যাশনাল বুক ট্রাস্ট, ইন্ডিয়া, নয়া দিল্লী, ১৯৮২, পৃ. ১১২ - ১১৪।

২. ড. মহীতোষ গায়েন, ড. সমর কান্তি চক্রবর্তী ও কৌশিক দত্ত (সম্পাদিত), 'বাংলার সমাজ ও সংস্কৃতি', রূপালি পাবলিকেশন, কলকাতা, ২০২০, পৃ. ৪৭।

৩. বিনয় ঘোষ, 'পশ্চিম বঙ্গের সংস্কৃতি' (১ম খন্ড), দীপ প্রকাশন, কলকাতা, ২০০৭, পৃ. ৫৩।

৪. দিলীপ কুমার গোস্বামী, 'মানভূম-পুরুলিয়া', ব্রজভূমি প্রকাশন, ২০০৭, পৃ. ১৬৮-৬৯।

৫. ছৌ-নাচের ইতিকথা, বর্তমান পত্রিকা, (মৃণ্ময় চন্দের বিশেষ প্রবন্ধ), ৪ ঠা অক্টোবর ২০২০।

৬. দিলীপ কুমার গোস্বামী, পূর্বোক্ত, পৃ. ১৬৮-১৭০।

৭. স্বপন কুমার কর্মকার বিশেষ সাক্ষাৎকার, গম্ভীর সিংমুড়ার নিজ বাসভবনে, চড়িদা, বাঘমুন্ডি, পুরুলিয়া, ১৯৯০।

৮. স্বপন কুমার কর্মকার, গম্ভীর সিংমুড়ার সঙ্গে সাক্ষাৎকার, এটি প্রকাশিত হয় 'বাংলার আভাষ' পত্রিকায়, ১৯৯১।

৯. গম্ভীর সিংমুড়ার বিদেশে ছৌ-নৃত্য প্রদর্শনের স্থান ও সালের নাম তারই মর্মর মূর্তির প্রস্তর ফলকে খোদাই করা আছে।

১০. স্বপন কুমার কর্মকার, গম্ভীর সিংমুড়ার সঙ্গে সাক্ষাৎকার, ১৯৯৫ (International Famous Adibasi Chhow Dance Party, Prop. Padmshree Gambhir Sing Mura. এই প্যাডটি আমাকে (স্বপন কর্মকার) তাঁর পুত্র শ্রীযুক্ত কার্তিক মুড়া তাঁর পিতার সম্পর্কে বিশেষ গুরুত্বপূর্ণ তথ্য লিখতে অনুরোধ করেন। আমি তা সংক্ষেপে লিপিবদ্ধ করি।

১১. তরুণ দেব ভট্টাচার্য, 'পুরুলিয়া', সেকাল-একাল, ফার্মা কে.এল.এম. প্রা লি, কলকাতা, ১৯৮৬, পৃ. ৩০৯।

১২. স্বপন কুমার কর্মকার, পূর্বোক্ত, 'বাংলার আভাষ' পত্রিকায়, ১৯৯১।

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