Evidences of Manure Driven and C:N Regulated Enhanced Carbon Status and Microalgal Productivity in Managed Aquatic System under Simulated Green House Conditions

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Abstract

The impact of greenhouse gas mediated raised temperature and carbon dioxide on the manure driven carbon status and algal primary productivity was examined in managed aquatic system using six different treatments: (i) cattle manure + saw dust, (ii) poultry dropping + saw dust, (iii) vermi-compost + saw dust (iv) mixed combination with cattle manure, poultry droppings, vermi-compost and saw dust, (v) iso-carbonic states maintained with cattle manure using vermi-compost and (vi) poultry droppings as basal dose maintained under open and simulated greenhouse conditions. There was an opposite responses of the time scale variations of buffering mechanisms between the closed and open conditions. The maximal carbon burial rate, organic carbon but reduced algal productivity in the mixed treatment and the reverse response in the poultry dropping and saw dust combination regulated by the C/N ratio of input manure revealed that the carbon sink in the bottom soil was primarily due to allochthonous origin in the former, and from autochthonous source in the later. It may be concluded that substantial rise in carbon status in the sediment due to greenhouse effect was attributable to enhanced microalgae derived allochthonous carbon sink mediated through integrative functions of temperature induced microalgal productivity and microbial driven manure decomposition. The dead microalgae and residual manure that settled down in the bottom surface enhanced the carbon status of the system, in question.

Keywords: Carbon status; Microalgal productivity; Aquatic system; Polyhouse; Soil-carbon; Simulated green house

Introduction

The climate change is now a scientific reality. The increasing concentrations of greenhouse gases such as carbon dioxide, carbon monoxide, methane and nitrous oxide are the main drivers of the global warming and climate change. The global warming potential of N₂O is 310 times more than CO₂ over a hundred year life span [1]. In India alone, agriculture sector contributes around 17.6% to the total annual anthropogenic emissions of greenhouse gases [2]. As a result, there has been a widespread international movement in order to reduce the emission of greenhouse gases by adopting several climate resilient mitigation and adaptation strategies.

The processes of biological carbon sequestration in the aquatic system framework involves trapping of atmospheric CO₂ through air-water CO₂ exchange, sequestration in water column via microalgae and carbon sink in bottom sediments. Biological CO₂ fixation has been extensively investigated as part of efforts to solve the global warming problem [3]. As microalgae consume high quantities of CO₂ to produce different types of biomass, algae based carbon capture storage technologies are being extensively researched using different strains of microalgae isolated from different habitats [4-6]. The carbon capture potentials of microalgae has been estimated to be about 1.83 kg CO₂ per kilogram microalgae [7] and microalgae or protoplankton support about half of the global primary production [8].

Because of the vast oceanic area, marine phytoplankton has the noble capacity to trap ~50% of the CO₂ that is fixed annually worldwide [9] by sequestering more than 100 million tons of carbon dioxide every day [10]. Microalgae are also cultivated commercially for production of biofuels, biodiesel, etc [7]. The advantages of culturing microalgae in inland and marine sources are: (i) no competition of space for agriculture (ii) harvested algae can be converted into different byproducts such as biodiesel, healthy food, animal feedstock, biogas and fertilizer, etc [6,11].

Wetlands are known to have mitigation effects through their ability to sink carbon, and adaptation effects through their ability to store and regulate water [12]. Wetlands, covering 6% of the world’s land surface and 12% of the global carbon pool, play a crucial role in the global cycling of carbon. The wetland soils containing nearly 14.5% of the world’s soil carbon are an important source for carbon sink [13,14] and contribute to the regional and global climate [15,16] by storing as high as approximately 44.6 Tg C yr⁻¹ globally [17]. Intensification of functional activity in managed wetlands particularly in aquaculture units has made them more dynamic having regular input of allochthonous matters in the form of fertilizers, manure and feed. A portion of feed lot, manure, and dead plankton settles in the bottom surface in the form of organic matter and eventually become a part of the soil in the long run. The process of humic formation in bottom surface of water bodies is considered a form of accumulation and storage of carbon containing compounds.

The status of carbon sink or sources affects the biodiversity of freshwater ecosystem across most of the Arctic, and the magnitude, extent and duration of the impacts have been projected to system-and location dependent [18]. It is reported that the global emission of carbon dioxide from inland waters are similar in magnitude to the carbon dioxide uptake by the ocean and that global burial of organic

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carbon in inland water sediments exceeds organic carbon sequestration on the ocean floor [19]. Thus, inland waters play even a much important role than ocean in terms of carbon sink via active microbial processes of carbon pump that are responsible for transformation of labile dissolved organic carbon into recalcitrant dissolved organic carbon in the bottom sediment [20]. The interplay between dynamic environmental conditions and metabolic responses determine resource allocation and biogeochemical cycling in aquatic system [9].

Aquaculture ponds sequester carbon as much as 0.21% of the annual global C emissions or about 10 Pg/year (1 Pg = Pentagram = billion ton). It is estimated that about 16.6 million tons of carbon is annually buried in aquaculture ponds worldwide [21] of which maximum sequestration occurs in Asia and particularly in China [22]. At the same time, one of the very strong greenhouse gas N\textsubscript{2}O-N has been projected to increase by 5.72% in 2030 (3.83 \times 10^{11} g) from its estimated emission of 9.30 \times 10^{10} g in 2009 if the aquaculture industry continues to rise at the current annual growth rate of 7.10% and thereby posing a debate on the issue [1,23]. Nevertheless, the role of aquaculture pond bottom has been greatly emphasized in the global cycling of carbon [24] due to their burial efficiency of organic carbon (buried OC: deposited OC) which was up to 48% (mean) and that was related with the input of allochthonous organic matter in numerous arctic lakes [19,25].

In aquaculture practice, qualitatively different organic manure are used as source of fertilizer for enhancement of fish production via the grazing and detritus food chain that induces organic carbon production through autochthonous and allochthonous sources. Organic matters from dead plankton, organic fertilizers, uneaten feed and excrement of cultured fish sink to the pond bottom and gradually mix with soil particles over time. The small impoundments in farming areas may bury more carbon than the world’s oceans [26,27]. This was due to intensification of farming activity per unit area compared to oceans. However, the role of manure driven carbon status in the framework of aquatic system and its state-of-the-art under greenhouse condition has hardly been researched.

It is hypothesized that greenhouse mediated raised temperature would influence the decomposition of chemically and qualitatively different manure that have immense potentials of carbon sink and bring out changes in the structure and functions of aquatic system through explicitly the involved processes of carbon sequestration such as air-water CO\textsubscript{2} exchange C-sequestration by microalgae and carbon sink in the sediment operating through integrative process of environmental, trophic and microbial activities for carbon pump. The purpose of the present study was to examine the manure driven primary productivity and carbon status in a simulated system under closed and open system within the framework of aquatic environment. The study is pertinent as it envisaged ascertaining the potentials of carbon burial rate of qualitatively different manure mediated through primary production and sink of organic matter as affected by the greenhouse effect.

Materials and Methods

Experimental design

The experiment was performed during winter (December, 2013 through March 2014) using thirty six experimental tanks (300 l) that are placed in equal numbers inside and outside the enclosed polyhouse simulating the green house condition. All the experimental tanks were provided with 18 cm soil at the bottom, filled with aerated ground water (pH 7.2-7.4) and allowed for a week for establishment. The constructed polyhouse with conventional bamboo structure and standard transparent polythene mimicked greenhouse functions.
Soil carbon burial rate

The soil carbon burial rate estimated from sediment accumulation rate, dry bulk density, and percentage organic C in sediment following the calculation as follows:

Soil carbon burial rate = Soil sedimentation rate × Soil bulk density ×% of Soil organic carbon

Climatic regime

Ambient air temperature, light intensity and atmospheric CO₂ were recorded from within and outside the polyhouse using digital thermometer, LUX meter and CO₂ meter (Lutron GCH-2018), respectively.

Statistical analysis

All the data were statistically evaluated; analysis of variance in the form of split plot model was applied to find the effects of experimental conditions (open and closed), treatments, time and interactions, if any. In this model, individual tanks were considered as whole plot treatment and time as subplot. This was followed by LSD test at 5% level.

Results

Light intensity and temperature

The mean intensity of light inside the polyhouse was attenuated by 200% with the values ranging from 145 to 340 LUX against 198 to 580 LUX in the open air system. The air temperature has been increased inside the polyhouse (34-44°C) compared to open air system (25-35°C) as a result of emission of greenhouse gases. The overall mean air temperature was 39°C and 29.5°C, in the closed polyhouse and open system, respectively.

The magnitude of temperature difference was less in water (5.23°C) than in air (9.5°C). Clearly, differences in thermal regime of water were responsible for microbial induced manure decomposition and subsequently productivity of microalgae and their potentials to sequester carbon in the system.

Air carbon dioxide

There has been significant rise in the concentrations (70%) of atmospheric carbon dioxide inside the polyhouse (490-905 ppm) compared to open air system (370 -440 ppm). The mean concentration of air carbon dioxide was 698 ppm in the enclosed polyhouse and 405 ppm in open air, respectively. The elevated mean atmospheric carbon dioxide inside the polyhouse affected the carbon dioxide level of water through absorption and brought about changes in buffering capacity of water as well as carbon sequestration potentials of photosynthesis of microalgae.

Primary productivity of microalgae

The primary productivity of phytoplankton experienced around 10-fold variations ranging from 32.33 mg/cm²/hr to 307.88 mg/cm²/hr in different treatments employed. Application of poultry droppings mixed with saw dust (T-2) resulted in highest gross primary productivity (GPP) (Figure 1), net primary productivity (NPP) (Figure 1) and community respiration (CR) of phytoplankton (Figure 1), and lowest in the treatment (T-4) with mixed manure (F₁₋₅ > 24.46; P < 0.001). LSD test revealed that three treatment groups were clearly recognized: highest in T-2, second in T-3 and T-4 and the lowest in the group comprising T-1, T-4 and T-6 (Figure 1).

Frequency distribution of gross primary productivity of phytoplankton (Figure 2), NPP (Figure 3) and CR (Figure 4) showed the occurrence of highest values in T-2 (poultry droppings and saw dust) as evident from more occurrence of higher class (250-279.99 mg/cm²/hr). Whereas such higher class range was absent in the remaining treatments (Figure 5). As a result, this group was significantly different (P < 0.05) from the next lower group (T-3 and T-4). The third group comprising T-1, T-4 and T-6 (Figure 5) that remained significantly different (P<0.05) from the rest two groups was characterized by the absence of productivity class range of 130.00-159.99 mg/cm²/hr. Responses of net primary productivity of microalgae and community respiration followed the trend similar to that of gross primary productivity.

Temporal courses of variations of GPP experienced almost a sigmoid curve in three groups of treatments. There was no difference in the values of GPP among the treatments during the initial lag phase, but the changes were well marked during the next logarithmic phase (Figure 1). Though there was no strong impact of green house (polyhouse) mediated raised temperature on specific treatment, the mean value for all treatments under simulated greenhouse condition remained significantly higher (P<0.05) than outside treatments. This shows that GHG emitted raised temperature was responsible for induction of manure driven primary production via microbial decomposition of manure and phytoplankton derived carbon sink in T-2.

Carbon burial rate

Carbon burial rate was less variable (3.57 fold) under simulated greenhouse condition ranging from 33.46 g/m² to 118.03 g/m² per 100 days compared to open system (23.81 g/m² to 102.94 g/m² per 100 days) in all treatments. Hence, the overall mean for different treatments attributable to greenhouse effects was 27% higher than under open conditions (Figure 6).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Treatments of manure</th>
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<tbody>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>Manures</td>
<td></td>
</tr>
<tr>
<td>900 g cow dung + 100 g saw dust</td>
<td>900 g poultry dropping + 100 g saw dust</td>
</tr>
<tr>
<td>Total amount of manure added (g)</td>
<td>1.0</td>
</tr>
<tr>
<td>Total input of carbon (g)</td>
<td>331.61</td>
</tr>
<tr>
<td>Total input of nitrogen (g)</td>
<td>15.41</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>21.52</td>
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</table>

Table 1: Manuring conditions followed in the experiment.
Treatment-wise, application of mixed manure (T-4) resulted in highest carbon burial rate among all the treatments under both the conditions examined. Reduced rates of carbon burial in cattle manure (T-1), poultry droppings (T-2) and iso-carbonic state with poultry dropping (T-6) implied that these treatments were less effective as a depository of carbon due to fast turnover of carbon in the cattle dung manure through biogeochemical cycling of decomposition and mineralization. However, the rate of increase was significantly higher in all treatments under closed system than in open ones (Figure 6) implying that increase of carbon sink was explicitly GHG dependent.

Water and soil environment

The pH-free CO$_2$ - CO$_3$ - HCO$_3$ system: Responses of pH to treatments were quite different between the enclosed and outside polyhouse. Fluctuations of water pH were more wide (7.87-8.09) inside the polyhouse compared to outside (8.47-8.80). Prevalence of more acidic condition of water inside the polyhouse than outside (P<0.05) was due to rapid rate of microbial decomposition of manure in the former caused by polyhouse mediated raised temperature (Table 2). The carbon dioxide- carbonate-bicarbonate systems also differed (ANOVA; P<0.001) between the two conditions employed. The concentrations of bicarbonate, total alkalinity and hardness of water were significantly higher (P<0.05) in the treatment with poultry dropping and saw dust (T-2) under closed system, whereas lower values were registered in the treatment with cattle manure and saw dust (T-1) under any condition either. This suggests that saw dust and poultry dropping combination caused alkaline state with more calcium carbonate and concomitant increase in the concentration of total alkalinity of water.

Dissolved oxygen (DO): The concentration of dissolved oxygen of water ranged from the minimum of 0.8 mg/l to the maximum of 10.9 mg/l in all the treatments during the course of investigation. Lowest values of dissolved oxygen in the poultry dropping and saw dust combination (T-2) reflected the maximum decomposition potential of this combination under both closed and open system.

Soil pH: Application of manure resulted in relatively reduced pH of soil with values ranging from 7.1-7.6 in open conditions against 6.9-7.6 in closed systems. Green house mediated enhanced microbial decomposition was responsible for the more acidic conditions which occurred in more than 84% of the total number of observations (Table 3).

Soil sedimentation rate: There was no difference in the rate of sedimentation ranging from 0.4 cm to 0.8 cm/100 days in different treatments employed under closed and open systems. The highest rate was observed in mixed treatment (T-4) both in closed and open systems. This was followed by vermi compost and saw dust (T-3) treatment showing the next higher values.

Organic carbon (OC): The organic carbon content of soil ranged from 11.52 mg C/g to 55.1 mg C/g and 16.08 mg C/g to 48.2 mg C/g in the treatments under closed and open system, respectively. The values were significantly higher (P<0.01) in all treatments under enclosed polyhouse than outside. Treatment-wise, mixed manure (T-4) and 801.94-673.38 µS/cm) showing almost similar responses among themselves.

GHG mediated raised temperature in T-2 caused 25 or 27% rise in the values of TDS (691.19 mg/l) and Specific conductance (954.88 µS/cm) compared to open system (TDS -552.69 mg/l; SC-753.06 µS/cm) As expected, specific conductivity measuring the ionic content of a solution was highly related with the TDS reflecting the biological productivity of the system in question.

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Figure 2: Frequency distribution of gross primary productivity (GPP) of phytoplankton against different class range of values using all treatments together.
Figure 3: Frequency distribution of net primary productivity (NPP) of phytoplankton against different class range of values using all treatments together.
Figure 4: Frequency distribution of community respiration (CR) against different class range of values using all treatments together.
iso-carbonic state with poultry dropping (T-6) showed the highest and lowest values, respectively (Table 3). On a temporal scale, increase in the concentration of organic carbon was registered over the passage of time regardless of treatments either in closed or open conditions. However, the rate of increase was found to be much higher under green house condition than outside. The variations of organic carbon content of accumulated sediment were almost similar to that of treatment related organic carbon of soil, being maximal in T-4 followed by T-3.

Discussion

Since the primary productivity of phytoplankton and soil carbon status have been significantly increased in all the tanks regardless of treatment under simulated greenhouse conditions than outside, it is apparent that increased carbon status related with carbon burial rate in the former was the result of manure driven algal productivity and rapid microbial decomposition and subsequent mineralization through biogeochemical cycling bacteria.

Algae inhabit a dynamic photic zone that changes both in quantity and quality of light and posses adaptive mechanisms with high degree of diversity in the photosynthetic apparatus to maximize light harvesting and to limit damage by excessive amount of photosynthetically active radiation and UV radiation in the environment. The green house mediated raised temperature (5.23°C) consequent upon the elevated atmospheric levels of CO₂ (490-905 ppm) under the enclosed polyhouse was responsible for the enhancement of primary productivity of phytoplankton mediated through dissolution of atmospheric CO₂ into water as well as availability of adequate nutrients released from autochthonous and allochthonous sources. Alternatively, this was explained as a result of prevalence of conducive range of both high temperature (34-44°C) and low light intensity (145 to 340 LUX) for the growth of microalgae or reduced photosynthetic performance of microalgae outside due to the effects of high light intensity (198 to 580 LUX) and low temperature (25-35°C). The effect of elevated levels of CO₂ on the photosynthetic performance of microalgae is well understood [33]. The role of light on the photosynthetic efficiency of phytoplankton has been tested by developing an exponential equation model to describe photosynthetic rate of phytoplankton as a function of light intensity for a variety of unicellular microalgae and higher plants where photosynthesis is proportional to \(1 = e^{-\beta I}\). The parameter \(\beta = I^{-1}\) is derived by simultaneous curve fitting method where \(I\) is incident quantum-flux density [34]. Chen [35] demonstrated that the optimal, minimal and maximal growth temperatures of phytoplankton decreases with latitude for marine taxa, whereas the minimal and maximal growth temperatures are relatively invariant with latitude for freshwater phytoplankton. Further the thermal breadth is larger for freshwater than marine phytoplankton. Using a mechanistic approach, Huertas et al. [8] provided evidence for different degrees of tolerance to temperature rises as well as an interspecific capacity for genetic adaptation. It is, therefore, reasonable to assume that the enhancement of carbon status in the tank sediment of all treatments under simulated greenhouse conditions was due to greenhouse effect. Using polyhouse experiment in fish ponds, Jana et al. [36] concluded that the benefit of closed polyhouse may be profitably used by the farmers for inducing breeding in tilapia and growth enhancement in other Indian fish during winter months and other region of the country in general.

The ideal C/N ratio for composting is generally considered to be around 3:1 under terrestrial conditions [37], whereas it was 11.8:1 for microbial decomposition of fertilizer in aquatic system [38]. About two fold variations of the C/N ratio of different input manure combinations (10.61 to 21.52) used in study reflected their potentials to sequester or source of carbon through microalgal dominated autotrophic or bacterial dominated heterotrophic metabolism in the framework of aquatic system. Accumulated organic matter reflecting organic carbon in the sediment is mineralized to CO₂ or CH₄ by heterotrophic microbes or it is gradually mixed with soil particles or buried in the sediment over time scale. Microbial carbon pump was found to exert a profound role in the transformation of labile organic carbon into recalcitrant organic carbon in the soil [20]. In aquaculture ponds, a layer of sediment with higher concentration of organic carbon of pond soil developed a characteristic profile of well defined strata or horizons [39].

The organic carbon content of soil ranging from 29.58-118.02 ppm was highly correlated \((r = 0.82; P < 0.001)\) with the carbon burial rates which varied by a factor of 5 in different treatments employed. Further, the green house effects were clearly manifested in the study because of distinctly higher values of soil organic carbon in all treatment tanks inside than outside.

Figure 5: Trend curves of average data for three distinctly different groups of treatments: T-2, (T-3 + T-5) and (T-1 + T-4 +T-6). The groups were significantly different from each other, whereas the treatments within the group were not.

![Image](image.png)
The primary productivity of microalgae was found to be substantially higher within the range of 25 and 40 mg/g of organic carbon of soil in the overall range of 11.52 and 55.1 mg in all treatments. This suggests that this range of organic carbon was conducive to microalgal production regardless of the condition employed. Further, the highest algal productivity accompanied by attenuated carbon microalgae production regardless of the condition employed. This suggests that this range of organic carbon was conducive to the blend of extremely high C/N ratio of saw dust (100-500) and low C/N ratio of poultry dropping (T-2) treatment combination and the reverse response in the mixed treatment (cattle manure, poultry dropping, vermi-compost and saw dust combination), implied that high manural value of the former treatment (cattle manure, poultry dropping, vermi-compost and saw dust combination), implied that high manural value of the former which was due to the blend of extremely high C/N ratio of saw dust (100-500) and low C/N ratio of poultry dropping (<10). This shows that the saw dust- almost a discarded material, can be effectively used as manure in aquaculture system in combination with poultry dropping even though the saw dust is rich in carbonaceous substances having very high C/N ratio (fresh-saw dust-200, decomposed saw dust-500). However, it is reported that there was no increase in soil organic matter and soil total carbon due to application of saw dust in agricultural corn field [40].

Substantial decline in carbon burial rate and organic carbon of soil in poultry dropping and saw dust combination was attributed to enhanced manure decomposition due to reduced carbon relative to nitrogen (C/N = 10.61), mineralization and subsequent utilization by microalgal biomass through biogeochemical cycling rather than its sink as residual organic carbon in sediment which was encountered in the mixed treatment (T-4) showing the reverse response with T-2 where nitrogen is more relative to carbon (C/N = 15.77). So, the overall soil carbon status in the former was primarily regulated by CN ratio driven autochthothonous source derived from dead massive algal bloom and less by the allochthothonous source of residual manure and vice versa in the later where heterotrophic metabolism and carbon sink dominated by the integrative environmental, trophic and microbial carbon pump. The lowest carbon burial rate in iso-carbonic state (T-6) using reduced load of poultry dropping and saw dust indicated that the carbon sequestrating capacity of the bottom soil was to some extent input load dependent even with low CN ratio (13.96).

Autochthothonous DOC accounted for >25% of the total water column DOC pool, and autochthothonous OC production attenuated...
the concentration of dissolved inorganic carbon in the water column and thereby contributing to atmospheric carbon dioxide uptake [41]. It is known that sediment metabolism is dependent upon the allochthonous carbon source [42], and much of the organic matter of autochthonous origin derived from dead plankton, fish excrement, etc. is readily decomposed, mineralized and utilized for ecosystem functioning. Lakes are grouped into lakes with net consumption of organic carbon (SOC) pool in soils could offset emissions of CO₂ from fossil fuel combustion [21].

As a mechanistic model, it is proposed that substantial rise in carbon sink due to greenhouse effect in the framework of managed aquatic system is attributable to microalgae derived autochthonous carbon sink mediated through integrative functions of temperature induced microalgal productivity and microbial driven manure decomposition. The dead microalgae and residual manure that settled down in the bottom surface enhanced the carbon status of the system, in question.

Table 3: Range and mean (± SE) of soil quality parameters analyzed in different treatments under enclosed polyhouse and open conditions.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Close</th>
<th>Open</th>
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</thead>
<tbody>
<tr>
<td>Soil pH</td>
<td>T-1 T-2 T-3 T-4 T-5 T-6</td>
<td>T-1 T-2 T-3 T-4 T-5 T-6</td>
</tr>
<tr>
<td>6.9-7.1</td>
<td>7.4-7.7</td>
<td>7.3-7.7</td>
</tr>
<tr>
<td>Water holding capacity (%)</td>
<td>69.099</td>
<td>71.543</td>
</tr>
<tr>
<td>Water sedimentation rate (cm/100days)</td>
<td>0.500 0.500 0.800 0.800 0.700 0.400</td>
<td>0.400 0.400 0.700 0.800 0.600 0.400</td>
</tr>
<tr>
<td>Bulk density (gm/cm³)</td>
<td>0.639 0.747 0.771 0.847 0.826 0.742</td>
<td>0.640 0.732 0.757 0.833 0.797 0.730</td>
</tr>
<tr>
<td>Total porosity (%)</td>
<td>57.921 53.416 57.522 60.871 58.240 59.715</td>
<td>55.649 55.210 55.848 60.711 59.276 59.475</td>
</tr>
<tr>
<td>Particle density (gm/cm³)</td>
<td>1.518 1.603 1.814 2.164 1.977 1.841</td>
<td>1.802 1.443 1.634 1.715 2.120 1.957</td>
</tr>
</tbody>
</table>

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