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ATP levels and adenylate energy charge in soils of mangroves in the Andamans

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Adenosine 5′-triphosphate (ATP) is considered to be a useful indicator of life in soil and the adenylate energy charge (AEC) indicates the energetic status of soil microorganisms. ATP concentration and AEC levels have been extensively studied in a diverse group of soils. However, little knowledge is available on the levels of ATP and AEC in soils of mangroves. We report here the levels of adenylates ATP, adenosine di-phosphate (ADP) and adenosine monophosphate (AMP)) and AEC in soils of undisturbed mangroves of South-, Middle-, North- and Little-Andamans. Relevant soil physico-chemical and microbial parameters and their relationship to ATP and AEC were also examined. Averaged across various mangrove sites, total N level was 1.44 ± 0.13 g kg^{-1}, organic C 15.6 ± 1.5 g kg^{-1}, microbial biomass C 410 ± 35 μg kg^{-1}, microbial biomass N 34 ± 2 μg kg^{-1} and qCO_2 41.1 ± 4.4 mg CO_2 (g biomass C)$^{-1}$ d^{-1}. Among the adenylates, ATP ranged

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from 2.32 to 3.22 nmol g^{-1} (mean 2.87 ± 0.29), AMP from 0.21 to 0.29 nmol g^{-1} (mean 0.25 ± 0.03) and ADP from 0.41 to 0.48 nmol g^{-1} (mean 0.44 ± 0.03). Across sites, the average microbial biomass C/organic C ratio was 2.6 ± 0.2% and microbial biomass C/N ratio at the mangrove sites was wider and ranged from 11.2 to 14.5 with a mean of 12.0 ± 0.9. The ATP/microbial biomass C ratio ranged from 6.0 to 8.2 μmol g^{-1} with a mean of 7.0 ± 0.6 μmol g^{-1}, markedly lower than the worldwide average of 10–12 μmol g^{-1} reported in a wide range of soils. Lower ATP/microbial biomass C ratio in our mangrove soils is most likely due to a changed microbial community structure indicating a decomposition pathway dominated by fungi and microorganisms with large microbial biomass C/N ratio. The AEC levels were consistently >8.0 (mean 0.87) at all the sites, suggesting that the majority of microorganisms in these mangrove soils are probably dormant.

Keywords: Adenylates, ATP, ATP/microbial biomass C ratio, adenylate energy charge, mangrove forests.

ADENOSINE 5′-triphosphate (ATP) occurs in all living cells[1], but exocellular ATP has a half-life of less than 1 h. The ATP content is, therefore, considered a useful indicator of life in soil[2]. Besides, there is substantial evidence to suggest that the soil microbial biomass maintains an ATP concentration typical of microorganisms undergoing exponential growth *in vitro*[3,4]. However, the soil microbial populations are supposed to be predominant in a dormant state with low metabolic activity and low turnover rates[5]. It was proposed that the energetic status of soil microorganisms can be evaluated by determining the adenylate energy charge (AEC)[6]. In cultures of microorganisms *in vitro*, AEC values >0.8 indicate actively growing cells, values from 0.5 to 0.7 represent dormant cells that are incapable of biosynthesis, and values <0.4 occur only in dead or dying cells[7]. Pioneering work on adenylates (ATP, adenosine di-(ADP) and monophosphates (AMP)) and AEC in soils was done by Jenkinson and co-workers[8,9], as well as Brookes and co-workers[6,10]. Subsequently, Contin *et al.*[2] combined appropriate published data on ATP available up to 1996 and some of their own results in addition to Jenkinson's data[9] to reexamine the literature on ATP and microbial biomass relationships in a wide group of soils from the northern hemisphere, southern hemisphere and Japanese paddy-dryland crop rotation. Notably, this included soils under different management regimes encompassing arable, grassland and woodland soils. More recently, the literature base of ATP and biomass relationship was made larger and diverse by Joergensen and co-workers[4,11–13] through their excellent work on a wide group of soils of temperate and tropical ecosystems. Various other published data on adenylates in soils also exist[14–17].

However, information on adenylates especially ATP and AEC in soils under the mangroves is limited. Mangroves are one of the most unique and endangered ecosystems of the biosphere covering 60–70% of the tropical coasts, especially in India, Thailand, the Philippines, Malaysia, Indonesia, Bangladesh and Papua New Guinea. In India, mangroves occur mostly in the west coast (Kerala, Karnataka, Goa, Maharashtra, Gujarat, coral atolls of Lakshadweep islands), and east coast (Tamil Nadu, Andhra Pradesh, Orissa, West Bengal, and the Andaman and Nicobar Islands). Among these, mangroves of the Andamans are considered to be the most luxuriant[18], covering about 77,769 ha[18].

For the study, four undisturbed mangroves sites were selected from each district of the Andamans (10°30′–13°42′N lat. and 92°14′–94°16′E long.) and 20 random cores (0–15 cm, 7 cm Ø) were taken from each site. The soils were then sieved (<2 mm), and analysed for their moisture content. Sub-samples for the determination of organic carbon and total N were sieved to pass through a 0.5 mm mesh. Soil pH was determined in a 1 : 2.5 soil : water suspension, organic C by the Walkley Black method[19], total N by the Kjeldahl method[20], clay content by the pipette method[21] and cation exchange capacity (CEC) by the method of Gillman[22]. The microbial biomass C and N were estimated by fumigation-extraction[23] using a factor of 0.45 (ref. 24) and 0.54 (ref. 25) respectively. The adenylates (ATP, AMP and ADP) were estimated by the procedure of Dyckmans and Raubuch[26]. Dimethylsulphoxide (DMSO), Na_3PO_4-buffer (10 mM), EDTA (20 mM) and a nucleotide-releasing buffer (benzalkonium chloride containing 2 mM Mg-EDTA, 10 mM ammonium acetate and 20 mM THAM, pH 7.75 with acetate)[11] were used as extractants. The energy status of soil microorganisms was evaluated by determining AEC, which is defined as: AEC = (ATP + 0.5 × ADP/(ATP + ADP + AMP)[27]. The metabolic quotient (qCO_2) was determined by measuring basal respiration (CO_2 evolution) in moist soil samples adjusted to 55% of its water-holding capacity. Briefly, the samples were pre-incubated for 3 days at 20°C in the dark followed by measuring CO_2 production for another 3 days by trapping CO_2 in 0.05 M NaOH. CO_2 production was then measured by titration of the excess NaOH with 0.05 M HCl. The metabolic quotient was calculated using the formula: (μgCO_2-C evolved in 3 days g^{-1} soil)/(μg biomass C g^{-1} soil)/3 days × 1000 = mg CO_2–C g^{-1} biomass C per day[12]. All values reported are means of 20 determinations expressed in an oven-dry basis (24 h at 105°C).

The results (Table 1) revealed that soil pH varied in a narrow range of 5.20–6.05, clay between 19 and 27%, CEC between 212 and 268 μmol g^{-1}, total N between 1.31 and 1.83 g kg^{-1} and organic C between 13.9 and 19.8 g kg^{-1}. Among the microbial characteristics, microbial biomass C varied from 366 to 478 μg_c g^{-1} (mean 410 ± 35) and microbial biomass C/organic C ratio from 2.3 to 3.1% (mean 2.6 ± 0.2; Table 2). The biomass C constitutes up to 5% of total organic C[28]. However, ratios varying from 0.27 to 7.0% have been reported from soils

Table 1. Relevant physico-chemical properties of soils of various mangrove sites in the Andamans

Location	pH (1 : 2.5 H_2O)	Clay (%)	CEC ($\mu mol_c\ g^{-1}$)	Organic C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)
South Andaman					
Collinpur	5.21 (0.04)	19 (2)	216 (22)	14.6 (1.6)	1.37 (0.16)
Shoal Bay	5.32 (0.08)	23 (2)	231 (21)	19.8 (1.3)	1.83 (0.24)
Crikabad	6.04 (0.05)	24 (3)	212 (19)	14.3 (2.2)	1.32 (0.17)
Namunagarh	5.71 (0.09)	26 (2)	232 (21)	16.9 (1.9)	1.42 (0.11)
Middle Andaman					
Baratang	5.43 (0.05)	21 (4)	236 (24)	17.1 (2.7)	1.62 (0.14)
Kadamtala	5.62 (0.08)	24 (3)	248 (23)	14.6 (3.0)	1.31 (0.18)
Betapur	6.05 (0.08)	20 (3)	222 (24)	14.1 (1.7)	1.32 (0.12)
Nimbutala	5.27 (0.07)	24 (4)	253 (26)	14.2 (1.7)	1.38 (0.12)
North Andaman					
Kalighat	5.62 (0.05)	20 (3)	261 (23)	15.6 (2.1)	1.42 (0.21)
Austin Creek	5.36 (0.06)	21 (5)	268 (24)	16.2 (1.4)	1.51 (0.22)
R. K. Puram	5.42 (0.08)	23 (5)	221 (16)	16.5 (2.1)	1.50 (0.16)
Paschim Sagar	5.63 (0.08)	25 (3)	228 (14)	16.3 (2.5)	1.48 (0.16)
Little Andaman					
Nethaji Nagar	5.21 (0.05)	24 (3)	233 (21)	16.0 (1.6)	1.46 (0.13)
Harbinder Bay	5.20 (0.08)	26 (3)	241 (15)	13.9 (1.7)	1.28 (0.21)
Vivekanandapuram	5.62 (0.08)	24 (4)	262 (23)	15.3 (2.1)	1.41 (0.21)
Dugong Creek	5.56 (0.04)	27 (4)	260 (26)	14.6 (2.7)	1.43 (0.21)
Mean ± SD	5.52 ± 0.26	23.2 ± 2.3	239 ± 17	15.6 ± 1.5	1.44 ± 0.13

Values in parentheses indicate standard error of mean.

Table 2. Microbial properties of soils of various mangrove sites in the Andamans

Location	C_{MIC}[a] ($\mu g\ g^{-1}$)	N_{MIC}[b] ($\mu g\ g^{-1}$)	qCO_2 mg CO_2 (g biomass C)$^{-1}$ d^{-1}	C_{MIC}/organic C (%)	C_{MIC}/N_{MIC}
South Andaman					
Collinpur	366 (49)	32 (3.1)	39.6	2.5	11.4
Shoal Bay	462 (37)	39 (3.0)	47.2	2.3	11.8
Crikabad	444 (18)	37 (2.4)	51.2	3.1	12.0
Namunagarh	450 (18)	34 (1.8)	43.8	2.7	13.2
Middle Andaman					
Baratang	478 (37)	33 (2.0)	48.5	2.8	14.5
Kadamtala	402 (14)	36 (2.2)	38.8	2.7	11.2
Betapur	410 (23)	35 (1.9)	34.9	2.9	11.7
Nimbutala	371 (14)	32 (1.6)	41.0	2.6	11.6
North Andaman					
Kalighat	383 (21)	32 (2.4)	38.1	2.4	12.0
Austin Creek	412 (31)	35 (3.6)	37.9	2.5	11.8
R. K. Puram	414 (36)	32 (3.4)	39.4	2.5	12.9
Paschim Sagar	421 (24)	35 (2.7)	38.0	2.6	12.0
Little Andaman					
Nethaji Nagar	414 (39)	34 (2.5)	40.3	2.6	12.2
Harbinder Bay	374 (36)	33 (3.6)	40.6	2.7	11.3
Vivekanandapuram	384 (21)	34 (4.1)	38.3	2.5	11.3
Dugong Creek	370 (22)	32 (2.8)	40.2	2.5	11.6
Mean ± SD	410 ± 35	34 ± 2	41.1 ± 4.4	2.6 ± 0.2	12.0 ± 0.9

[a] C_{MIC}, Microbial biomass C; [b] N_{MIC}, Microbial biomass N; Values in parentheses indicate standard error of mean.

across different management systems, sampling times and analytical methods[29]. The microbial biomass N ranged from 32 to 39 $\mu g\ g^{-1}$ (mean 34 ± 2; Table 2). This is lower than the range (41–54 $\mu g\ g^{-1}$) reported under moist deciduous and semi-evergreen forests of the Andamans[17], but almost identical to the range (32–36 $\mu g\ g^{-1}$) reported under secondary tropical forest sites of the Philippines[12].

Among the adenylates (Table 3), AMP ranged from 0.21 to 0.29 nmol g^{-1} (mean 0.25 ± 0.03), ADP from 0.41 to 0.48 nmol g^{-1} (mean 0.44 ± 0.03) and ATP from 2.32

Table 3. Levels of adenylates (ATP, AMP, ADP), AEC and ATP/C_{MIC} ratio of soils of various mangrove sites in the Andamans

Location	ATP (nmol g^{-1})	AMP (nmol g^{-1})	ADP (nmol g^{-1})	AEC	ATP/C_{MIC} (μmol g^{-1})
South Andaman					
Collinpur	2.50 (0.14)	0.29 (0.04)	0.47 (0.08)	0.84	6.8
Shoal Bay	3.02 (0.12)	0.24 (0.06)	0.46 (0.08)	0.87	6.5
Crikabad	3.10(0.32)	0.24 (0.05)	0.48 (0.06)	0.87	7.0
Namunagarh	3.21 (0.21)	0.27 (0.05)	0.43 (0.04)	0.87	7.1
Middle Andaman					
Baratang	3.22 (0.18)	0.26 (0.04)	0.48 (0.04)	0.87	6.7
Kadamtala	2.52 (0.22)	0.28 (0.06)	0.48 (0.06)	0.84	6.3
Betapur	2.76 (0.14)	0.25 (0.06)	0.41 (0.07)	0.87	6.7
Nimbutala	2.43 (0.23)	0.28 (0.03)	0.42 (0.06)	0.84	6.5
North Andaman					
Kalighat	2.32 (0.17)	0.21 (0.02)	0.41 (0.05)	0.86	6.0
Austin Creek	2.98 (0.19)	0.25 (0.02)	0.42 (0.05)	0.87	7.2
R. K. Puram	2.84 (0.21)	0.25 (0.03)	0.43 (0.03)	0.87	6.8
Paschim Sagar	3.12 (0.09)	0.26 (0.05)	0.48 (0.07)	0.87	7.4
Little Andaman					
Nethaji Nagar	3.11 (0.13)	0.21 (0.05)	0.45 (0.07)	0.88	7.5
Harbinder Bay	2.82 (0.15)	0.22 (0.04)	0.41 (0.04)	0.88	7.5
Vivekanandapuram	3.17 (0.19)	0.25 (0.05)	0.41 (0.05)	0.88	8.2
Dugong Creek	2.81 (0.09)	0.21 (0.04)	0.43 (0.05)	0.87	7.6
Mean ± SD	2.87 ± 0.29	0.25 ± 0.03	0.44 ± 0.03	0.87 ± 0.01	7.0 ± 0.6

Values in parentheses indicate standard error of mean.

to 3.22 nmol g^{-1} (mean 2.87 ± 0.29). Average ATP levels are reported to be 4.2 μmol g^{-1} in grassland soils, 2.1 μmol g^{-1} in forest soils and 1.2 μmol g^{-1} in arable soils[16]. We also observed a positive correlation between biomass C levels and ATP ($r = 0.68$ at $P < 0.001$, $n = 160$) and sum of adenylates ($r = 0.65$ at $P < 0.001$, $n = 160$), which suggested that soils with greater biomass C levels are most likely to possess higher ATP levels. A similar relationship was observed in a large group of soils under different management regimes[2,13].

The mean ATP/microbial biomass C ratio (Table 3) was 7.0 ± 0.6 μmol g^{-1} (range 6.0–8.2; Table 3). This is markedly lower than the average value of 11.7 μmol g^{-1} and the geometric mean of 10.5 μmol g^{-1} reported in a wide range of soils of the northern and southern hemispheres under diverse management regimes[2,9]. It is pertinent to note that ATP levels in these studies were determined using the enzymatic luciferin/luciferase system. However, in recent studies wherein ATP was determined using the DMSO extractant, ATP/microbial biomass C ratio ranged between 3.1 and 5.2 μmol g^{-1} in secondary tropical forest sites[12], between 3.2 and 8.9 μmol g^{-1} in soils amended with glucose[4] and between 4.1 and 5.6 μmol g^{-1} in wet tropical forests of the Andamans[17]. In the present study also, ATP was determined using the DMSO extractant. The mean ATP/microbial biomass C ratio of 7.0 ± 0.6 μmol g^{-1} in our mangrove soils is close to the average value of 8.7 μmol g^{-1} observed in arable soils[30] and almost identical to the mean ATP/biomass C ratio of 7.1 μmol g^{-1} reported recently in a wide range of forests, grasslands and arable soils[13]. They have excluded the possibility of incomplete extraction of the added ATP to be the major reason for such lower ATP/microbial biomass C ratios because of the high extraction efficiency (between 90 and 95%) of the alkaline DMSO extractant. Therefore, the most plausible explanation for the relatively low average ATP/biomass C ratio in the mangrove soils compared to those of Jenkinson[9] and Contin *et al.*[2] could be differences in the soil microbial community structure[13]. Probably, the microbial community structure of our mangrove soils is dominated by fungi and microorganisms with large microbial biomass C/N ratio. The microbial biomass C/N ratio is considered to be an indicator of the relative proportion of fungi to bacteria[31]. Consequently, wider ratios (range 11.2–14.5, mean 12.0 ± 0.9; Table 2) indicate that fungi dominated these mangrove soils compared to bacteria. Similar observations were made in forest floor layer and acidic forest A horizon dominated by fungi[32] and microorganisms with a large biomass C/N ratio[33]. They[32,33] attributed this to higher C availability coupled with relatively low N availability due to which the production of enzymes involved in the metabolic pathways producing ATP is inhibited. Earlier reports on distribution of microorganisms in mangrove soils and waters from the Southern region along the coast of the Andaman Sea and the Gulf of Thailand, also indicate that fungi dominate bacteria and algae[34]. About 60 fungal species were identified, out of which *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp., *Fusarium* sp. and

Rhizoctonia sp. were found to be the most frequent in mangrove soils of the Andamans[35].

AEC, which indicates the energy status of soil microorganisms ranged from 0.84 to 0.88 (mean 0.87; Table 3). AEC levels ranging from 0.67 to 0.74 under secondary tropical forest sites of the Philippines[12] and those ranging from 0.85 to 0.87 under the moist deciduous and semi-evergreen forests of the Andamans[17] have been reported. Nevertheless, values ranging from 0.3 to 0.9 have been observed in a wide range of soils[6]. AEC levels >8.0, similar to those observed in our mangrove soils, have been described in soils where majority of the microorganisms are probably dormant[10,36]. It is also plausible that highly active microorganisms have large metabolic quotient (qCO_2) coupled with large ATP/biomass C ratios and high AEC[37]. However, no significant relationship between these three indices has been found in soils[13]. Though significant correlation between AEC and qCO_2 has been observed[36], the relationship between these two in our mangrove soils was non-significant. We also did not observe any significant relationship between AEC and individual parameters like pH, CEC, clay, organic C, total N, etc. Therefore, it is still not clear as to how soil microorganisms maintain such high AEC levels, similar to actively growing microorganisms *in vitro*[4]. Nevertheless, it has been hypothesized that the survival strategy of soil microorganisms is based on a resting population expending energy to maintain a state of metabolic alertness for immediate use of any exogenous substrate[38]. Therefore, an unknown combination of different factors like quantity and quality of soil organic matter, texture, pH, etc. seems to influence AEC levels in soils[39].

Overall, the mean ATP levels in our mangroves soils (0–15 cm) was 2.87 ± 0.29 μmol g^{-1} and the mean ATP/microbial biomass C ratio was 7.0 ± 0.6 μmol g^{-1}, considerably lower than the worldwide average of 10–12 μmol g^{-1} ATP g^{-1} biomass C observed in a wide range of soils[2,9]. This apparent discrepancy is most likely due to a changed microbial community structure possibly dominated by fungi and microorganisms with large microbial biomass C/N ratio. The mean AEC level of 0.87 is within the range of 0.3–0.9 observed in a large group of soils[6]. However, AEC values >8.0 indicate that majority of microorganisms in our mangrove soils are probably dormant. It also needs to be emphasized that the data presented are from soils sampled before the tsunami struck the shores of the Andaman and Nicobar Islands on 26 December 2004. Nevertheless, it can be presumed that variation in the adenylate and AEC levels between the pre- and post-tsunami soil samples would be minimum due mainly to the fact that mangroves are frequently inundated by sea water during periods of high tide. However, at sites that have been permanently submerged post-tsunami, variation in the levels of adenylates, ATP/microbial biomass C ratio and AEC from those reported by us is a distinct possibility.

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Biosorption of metals from contaminated water using seaweed

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Heavy metals are major pollutants in marine, lake and groundwaters as well as in industrial and even treated effluents. Biosorption, an inexpensive and reliable method to remove cadmium and lead ions from solution using dry seaweed biomass as adsorbents, was investigated. *Sargassum wightii* exhibited maximum metal uptake at pH 4–5 and the value ranged from 18% to 29% of dry biomass. The kinetics of metal adsorption was fast with 70–80% taking place within 30 min. Based on these results, a biobattery involving perforated columns packed with pulverized dry biomass of *S. wightii* was designed, which could remove metals in the range of 50–97% from a multi-metal ion solution within two and a half hours. The mechanism of metal sorption by seaweeds and the advantages of the present design of seaweed columns are discussed in the light of ecofriendly and cost-effective approach for effluent treatment.

Keywords: Biobattery, biosorption, effluent treatment, heavy metals, *Sargassum wightii*.

HEAVY metals can be extremely toxic as they damage nerves, liver, kidney and bones, and also block functional groups of vital enzymes[1]. Stringent environmental legislation and powers of the authoritative bodies established to enforce these regulations are increasing the demand for new technologies to remove metal from wastewater. For more than a decade, researchers have been looking for cheaper and more effective methods to remediate heavy metal-contaminated waters and reduce the growing public-health risk. Biosorption is proven to be quite effective at removing metal ions from contaminated solution in a low-cost and environment-friendly manner[2]. The major advantages of biosorption over conventional treatment methods include low cost, high efficiency of metal removal from dilute solution, minimization of chemical and/or biological sludge, no additional nutrient requirement, regeneration of biosorbent and the possibility of metal recovery[3].

Bacteria[4], fungi[5], marine algae[6,7], etc. have been studied for their heavy metal uptake capacities and suitability to be used as development of biosorbents. Biosorptive capacities of seaweeds, activated carbon and natural zeolites have been evaluated and are comparable to those of synthetic ion-exchange resins[6]. Marine macro-algae are harvested or cultivated in many parts of the world and are therefore readily available in large quantities for the development of highly effective biosorbent materials. This study inves-

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