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A preliminary study on some ecological factors involved in Mud-Puddling of butterflies in Mumbai metropolitan region

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Abstract
Butterfly mud puddling is a complex process which involves various factors. Present study deals about analysis of the abiotic factors that can have probable positive influence on puddling. Areas like Yeoor, Nagla and Conservation Education Centre (Bombay Natural History Society) of Sanjay Gandhi National Park, Tungareshwar Wildlife Sanctuary, Karnala Sanctuary, Matheran were visited (n = 21) in 2016 and 2017. Various abiotic factors were analysed like air temperature (avg. 30.21˚C), soil temperature (avg. 26.16°C), relative humidity (avg. 76.38%), temperature gradient between soil and air (avg. 4.05°C), soil pH (avg. 6.84), soil moisture (avg. 33.06%), sodium (avg. 0.54%), total nitrogen (0.19%). The different soil types recorded were – clay, sandy-clay loam, sandy clay, clay loam, sandy loam, loamy sand and loam.

Keywords: mud-puddling, butterflies, physical parameters, chemical parameters, Mumbai

1. Introduction
The phenomenon, which is most frequently shown by insects by forming aggregations, often by the hundreds, drinking at the edge of puddles, is appropriately termed as "Mud-puddling". Although puddling is most commonly observed in butterflies as it is a diurnal insect, the nocturnal insects like moths also do puddling. Mud-puddling is synonymous with the 'salt licking' performed by higher land vertebrates. It is thought that mud-puddling acts as a supplementary diet rather than as an energy provider. Some hypotheses were made on this behavior of adult Lepidoptera. Boggs, and Jackson, (1991) [6] hypothesized that "Individuals puddle to obtain scarce nutrients which are not obtained from their normal diet and also nutrient requirements vary from sex and age." and "Puddling is an alternative foraging technique may be because individuals are less successful to defend a foraging site." Arms *et al*., (1974) [2] hypothesized that "Males may have greater requirement for sodium in neuromuscular activity because they spend larger time in flight than females." and "Males must be collecting sodium and transferring it to females during mating." Sevastopulo (1959) [17] reported that the moisture in the soil attracts butterflies.
The minerals such as "sodium" ((Pivnick, and McNeil, (1987) [16]; Smedley and Eisner, (1996)) [19] and nutrients such as "amino acids" ((Arms *et al*., (1974) [2]; Boggs & Gilbert, (1979) [5, 6] obtained from puddles by males are transferred to females during mating, known as "nuptial gift". This nuptial gift along with sperm is transferred to the female via spermatophore (Drummond, 1984) [8]. The female then transfers these nuptial gifts in substantial quantities to the eggs (Smedley and Eisner, 1996) [19]. Study done by Arms *et al*., (1974) [2] and Adler and Pearson (1982) [1] shows that sodium ion stimulates the puddling behavior in males of *Papilio glaucus* Linnaeus, and established that amino acids obtained from the soil are incorporated into the body as proteins. Downes (1973) [7] reported butterflies feeding on mineral rich resources. Payne and King (1969) [9] and Shields (1972) [18] reported butterflies visiting pig and bob cat carrion respectively.
Amino acids have limiting effect on the breeding of herbivorous insects (Molleman, 2009) [14] consequently it can corroborate the fact that the puddling for nitrogenous compounds can increase fecundity. Hence butterflies aggregate on the urine of mammals and uric acid of birds, dead fish, and crustaceans. Sodium and nitrogen intake are correlated as McLean and Caveney (1993) [12] demonstrated that sodium helps in amino acid uptake.
India is one of the few countries having very high biodiversity owing to its unique geographical location. Almost all kinds of forest from intertidal forests to the alpine forests are found within a span of a few thousand kilometers. Almost throughout the distribution range of Indian butterflies, the mud-puddling phenomenon is very prominent and discrete. The mud-puddling behavior by Indian butterflies is chiefly observed from January to April.
Sanjay Gandhi National Park in Mumbai is home to 141 species of butterflies (Patwardhan, 2014) [15]. Around Mumbai, there are many protected forests which harbor almost similar Rhopaloceran diversity. There are many photographic records of butterflies aggregating on the mud in the forests in and around Mumbai comes under the Mumbai Metropolitan Region (MMR) of Maharashtra; thus, in the present study, MMR was selected as the study area.

2. Materials & Methods
The sampling sites (Table 1)
The sampling sites from MMR were Yeoor and Nagla block

of Sanjay Gandhi National Park; Pelhar dam on the outskirts of Tungareshwar Wildlife Sanctuary; a stream in Karnala Bird Sanctuary; Simpsons Tank, Vetaleshwar and Charlotte Lake of Matheran; and the artificially created stream at Conservation Education Center (Bombay Natural History Society), Goregaon (abbreviated as CEC).

The areas were visited during the puddling season for the two consecutive years 2016 and 2017 (February-March, 2016 & January-March, 2017). The observation time was from 09:00 AM in the morning to 14:00 PM. Soil samples were collected along the sides of the stream where butterflies were puddling. Each sample was packed in a separate airtight zip lock bag. These samples were further used for off-site analysis.

Table 1: Sampling sites & date

Sr.no	Area	Site	Dates
1	Yeoor	Y1	19/2/2016
2		Y2	19/2/2016
3		Y3	5/3/2017
4		Y4	5/3/2017
5		Y5	3/5/2017
6		Y6	13/3/2017
7		Y7	13/3/2017
8	Pelhar	P1	28/2/2016
9		P2	28/2/2016
10		P3	28/2/2016
11	Karnala	K1	15/1/2017
12		K2	15/1/2017
13	Matheran	M1	6/3/2016
14		M2	6/3/2016
15		M3	8/1/2017
16		M4	8/1/2017
17		M5	23/2/2017
18		M6	23/2/2017
19	Nagla	N1	23/1/2017
20		N2	23/1/2017
21	CEC	CEC	5/1/2017

Physical Parameters

The soil pH was measured using a pH meter (phep®), which was calibrated to pH 7 by Distilled Water.

The air temperature was measured at shoulder level (5ft above ground level) using simple mercury bulb thermometer. And the soil temperature was measured using the same thermometer by placing the bulb one inch under the soil surface. The temperature gradient between soil and air was also calculated.

The relative humidity was measured using standard dry and wet bulb method and calculated using standard charts.

For moisture content known quantity of wet soil was kept in a pre-weighed petri-dish for drying at 60°C in the oven till it showed constant weight.

For estimation of soil texture 100gm of wet soil was kept in a pre-weighed petri dish for drying at 60ºC in the oven. Dried soil was weighed and sieved using different mesh size sieves of ASTM No. 5, 8, 14, 25, 60, 80, 120 & 270 respectively. Each fraction of soil obtained was weighed separately and percentage of the same was calculated. Soil type was then determined by using the standard textural triangle chart. The soil fraction obtained from sieves of ASTM NO. 5 and 8 were Pebbles, where, the former mesh size sieved out the very fine pebbles & the latter one sieved out very fine granules of pebbles. The soil fraction obtained from sieves of ASTM No. 14, 25, 60, 80 & 120 were Sand, where, each fraction of sand sieved out by the above mentioned sieves were very coarse sand, coarse sand, medium sand, fine sand & very fine sand respectively. The soil fraction obtained from the sieve of ASTM No. 270 was Silt to be specific-coarse silt. The fraction of soil which passed the last sieve of ASTM No. 270 was considered as Clay.

Chemical Parameters

For analysis of Exchangeable Sodium percentage**,** 0.1 gm of dried soil sample was digested by acid mixture (6ml HNO_3, 2ml HCl, 2ml HF) in a micro-digester. The digested sample was used for sodium analysis using Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP – AES) (SPECTRO Analytical Instruments GmbH, Germany. Model: ARCOS, Simultaneous ICP Spectrometer) at SAIF lab of IIT, Bombay.

For analysis of Total Nitrogen content 5mg of dried soil sample was send to SAIF lab of IIT, Bombay; where, the analysis was done using CHNS (O) Analyzer (Thermo Finnigan, Italy. Model: FLASH EA 1112 series).

3. Results

Soil samples were tested for physical as well as for chemical parameters.

Physical Parameters (Table 2)

Soil pH

The pH range observed during the sampling period was 6.5 to 7.7 with average pH 6.84.

The pH at Yeoor, Pelhar, Nagla & CEC were slightly acidic ranging from 6.5 – 6.9 than the Karnala which shows basic pH (K1& K2). Matheran shows both the pH range i.e.; slightly acidic at four sites (M1, M2, M5 & M6) & basic at two sites (M3 & M4).

The lowest pH 6.5 was found at Y5, Y7 & M5 sites of Yeoor & Matheran. The highest pH 7.7 was found at M7 at Matheran.

Temperature: Air temperature and soil temperature was recorded.

Air Temperature

Overall minimum air temperature measured was 22°C at Matheran (M4) & maximum air temperature was 37°C at Yeoor (Y4). In the present study, average temperature obtain was 30.21°C.

In Yeoor, the air temperature varied 27.5°C (Y1) to 37°C (Y4). In Pelhar, the air temperature variation was from 28.5°C (P1) to 30.5°C (P3). In Karnala the air temperature was 27°C and 30°C respectively at both the sites (K1 & K2). In Matheran, the air temperature showed variation from 22°C (M4) to 33°C (M5).In Nagala, the air temperature was 34°C at both the sites (N1 & N2) showing no variation in temperature. In CEC, the air temperature was 29°C.

Soil Temperature

Overall minimum soil temperature measured was 16.5°C at Matheran (M4) & maximum air temperature was 31°C at

Nagla (N2). The mean soil temperature obtain was 26.16°C. In Yeoor, the soil temperature varied 22.8°C (Y2) to 30°C (Y4). In Pelhar, the soil temperature variation was from 25°C (P1) to 29°C (P3). In Karnala the soil temperature was 25°C & 26°C respectively at both the sites (K1 & K2). In Matheran, the soil temperature showed variation from 16.5°C (M4) to 28.5°C (M2). In Nagla, the soil temperature was 28°C& 31°Crespectively at both the sites (N1 & N2) showing variation of only three degree. In CEC, the soil temperature was 30°C.

Soil air temperature gradient

The soil gradient ranged between -1°C to 8°C with the average of 4.05°C.

At CEC the soil was 1°C hotter than the ambient air temperature and at station M2 in Matheran it was 0.5°C hotter. The soil at Yeoor was 3.25°C to 8°C hotter than the ambient air. Yeoor was the hottest of all stations.

Relative Humidity

Relative humidity ranged from 63% to 93% with the average of 76.38%. Pelhar (80 to 86%) and Karnala (85 and 93%) were the stations with the highest relative humidity. Yeoor showed highest variation (63 to 87%). The single sampling stations at CEC showed 68% relative humidity.

Moisture Content of Soil

Overall lowest soil moisture was 9.17% at Y7, Yeoor while the highest was 77.46% at M6, Matheran. The mean moisture content of the soil was 33.06%.

Moisture content of soil at Yeoor, Karnala & CEC was relatively low as compared to other areas. The highest moisture content of soil at Yeoor was 23.64% (Y1 & Y2) & lowest was 9.17% (Y7). The difference between the moisture content of soil at Karnala was 16.18%. At CEC, soil moisture was 18.76%. At Pelhar, the soil moisture of all the three sites was highest as compared to other areas, here, lowest moisture content was 50.59% (P3) & highest was 68.02% (P1). The highest moisture content at Matheran was 77.46% (M6) & lowest was 26.47% (M1). The soil moisture at sites M2, M3, M4 & M5 showed minimal variation. And most importantly, samples of site M1 (2016) & M3 (2017) belong to the same place at Vetaleshwar but with different sampling year; showed such a big difference (M1-55.64% & M3-26.47%) in the moisture content. This difference may be due to the reason that in 2016 water of the stream was more as compared to 2017. At Nagla, soil moisture decreased from 60.05% (N1) to 37.93% (N2).

Table 2: Physical parameters of the soils at sampling stations (except soil texture).

	Site	pH	Temperature (°C)			Relative Humidity (%)	Moisture (%)
			Air temp. (°C) (A)	Soil temp. (°C) (S)	Temp. Gradient (A-S)		
1	Y1	6.9	27.5	24.25	3.25	77	23.64
2	Y2	6.9	29	22.8	6.2	63	15.51
3	Y3	6.6	34	26	8	74	12.87
4	Y4	6.6	37	30	7	77	9.78
5	Y5	6.5	36	28	8	87	18.34
6	Y6	6.9	33	26	7	75	9.17
7	Y7	6.5	33	28	5	69	14.33
8	P1	6.8	28.5	25	3.5	86	59.75
9	P2	6.9	30	28	2	80	68.02
10	P3	6.8	30.5	29	1.5	80	50.59
11	K1	7.5	27	25	2	85	29.68
12	K2	7.3	30	26	4	93	13.53
13	M1	6.7	25.5	23.5	2	78	55.64
14	M2	6.9	28	28.5	-0.5	66	28.5
15	M3	7.7	23	20	3	78	26.47
16	M4	7	22	16.5	5.5	65	30.78
17	M5	6.5	33	28	5	80	33.49
18	M6	6.7	30.5	26	4.5	78	77.46
19	N1	6.6	34	28	6	65	60.05
20	N2	6.7	34	31	3	80	37.93
21	CEC	6.8	29	30	-1	68	18.76
Average		6.847	30.214	26.169	4.045	76.38	33.061

Soil Texture (Table 3)

The different soil types recorded were – clay, sandy-clay loam, sandy clay, clay loam, sandy loam, loamy sand and loam.

The type of soil found at Yeoor varied amongst all the sampling sites. At Y1, Y2 & Y7, sand was the major component of the soil type Sandy clay loam. At Y3 and Y6 soil type found was Sandy clay and Clayey respectively, whereas, at Y4 & Y5 possessed Loamy sand soil type. At Pelhar the soil type of sampling sites P1, P2 & P3 were Loamy & Sandy loam. The soil type of sampling sites K1 & K2 at Karnala was Sandy clay, thus, shows major presence of sand. Matheran shows predominantly sand as their major fraction of soil type - Sandy loam (M1), Sandy Clay (M2 & M5), Sandy clay loam (M3 & M4), except at M6 where clay is the major fraction of the soil. At Nagla, sampling sites N1 & N2 possessed Sandy clay loam & clayey type of soil. CEC showed presence of Sandy clay soil type.

Table 3: Soil Texture of the soils at sampling stations.

Sr. No.	Site	Soil Fraction (%)				Soil Type
		Pebbles Total %	Sand Total %	Silt %	Clay %	
1	Y1	10.67	87.59	1.63	0.11	Sandy clay loam
2	Y2	20.51	77.61	1.57	0.31	Sandy clay loam
3	Y3	10.61	89.01	0.38	0	Sandy loam
4	Y4	3.01	96.74	0.21	0.04	Loamy sand
5	Y5	8.35	90.65	0.95	0.05	Loamy sand
6	Y6	52.63	46.74	0.58	0.05	Clayey
7	Y7	13.52	84.79	1.52	0.17	Sandy clay loam
8	P1	6.31	56.96	34.29	2.44	Loamy
9	P2	0.69	52.81	43.06	3.44	Loamy
10	P3	4.39	69.17	24.79	1.65	Sandy loam
11	K1	14	73.16	11.78	1.06	Sandy clay
12	K2	48.51	49.73	1.76	0	Sandy clay
13	M1	1.52	86.62	11.07	0.79	Sandy loam
14	M2	35.37	59.3	4.96	0.37	Sandy clay
15	M3	22.99	73.67	2.97	0.37	Sandy clay loam
16	M4	17.5	79.07	2.99	0.44	Sandy clay loam
17	M5	39.33	57.12	3.26	0.29	Sandy clay
18	M6	21.32	45.7	30.75	2.23	Clay loam
19	N1	40.47	53.07	6.05	0.41	Sandy clay loam
20	N2	36.81	58.51	4.23	0.45	Clayey
21	CEC	37.35	61.5	1.08	0.07	Sandy clay

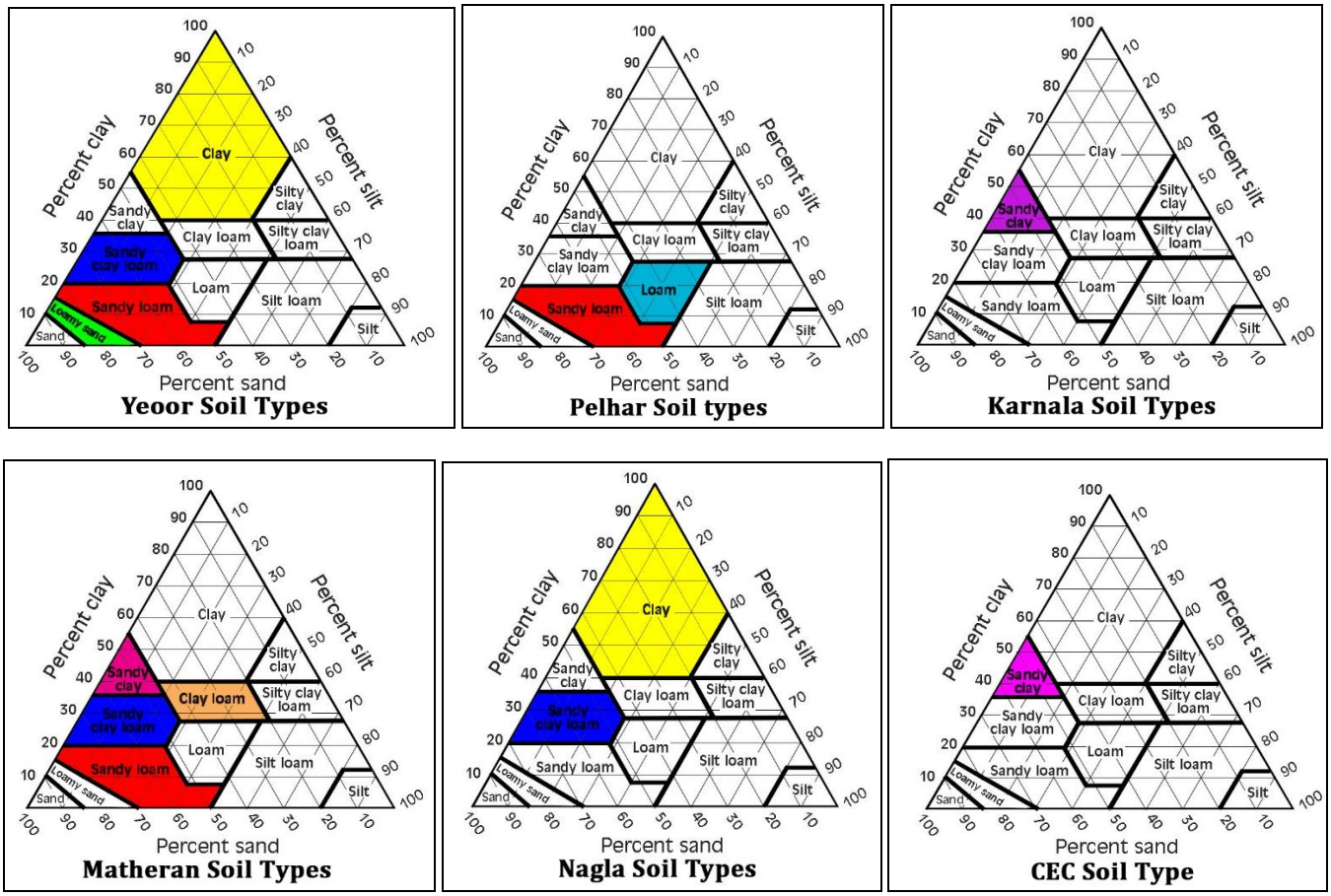

Fig 2: Soil Textural Triangle

Table 4: Dissolved Nutrients – Na and N

Site	Dissolved Nutrients	
	Na (%)	N (%)
Y1	0.453434	0.118
Y2	0.381817	0.159
Y3	0.737012	0.132
Y4	0.373208	0.126
Y5	0.553493	0.136
Y6	0.954922	0.172
Y7	0.951593	0.108
P1	0.783058	0.23
P2	0.980435	0.131
P3	0.760786	0.195
K1	0.305401	0.264
K2	0.523233	0.151
M1	0.15791	0.171
M2	0.487509	0.217
M3	0.293457	0.263
M4	0.292687	0.203
M5	0.159114	0.236
M6	0.26177	0.226
N1	1.085358	0.232
N2	0.379403	0.353
CEC	0.570949	0.285
AVG.	0.545074	0.195619

Chemical Parameters (Table 4)

Exchangeable Sodium Percentage

Overall maximum soil Na level was 1.08% at site N1, Nagla & minimum was 0.15% at sites M1 & M5, Matheran. The mean Na content of the soil was 0.54%.

Soil of N1 & N2 sites at Nagla showed major difference in the Na levels, i.e., 1.08% (N1) and 0.37% (N2). Followed by Nagla, Yeoor showed major variations in the soil Na levels; minimum soil Na level was 0.37% (Y4) and maximum was 0.95% (Y6 & Y7). The minimum variation in soil Na levels was seen at Pelhar and Karnala. At Pelhar, soil Na levels varied from 0.76% (P3) to 0.98% (P2). At Karnala, soil Na levels increases from 0.30% (K1) to 0.52% (K2). At Matheran, soil of M1 and M5 sites showed minimum Na level of 0.15% respectively, whereas, M2 showed maximum Na level of 0.48%; soil Na levels at M3, M4 and M6 were 0.29%, 0.29% and 0.26% respectively.

Total Nitrogen

Overall minimum soil N level was 0.108% at site Y7, Yeoor and the maximum soil N level was 0.353% at site N2, Nagla. The mean N content of the soil was 0.195%.

Soil N level at Yeoor showed minimum variation; the lowest value was 0.108% (Y7) & highest value was 0.172% (Y6). At Pelhar, soil N levels were 0.23% (P1), 0.131% (P2) & 0.195% (P3). At Karnala, soil N levels were 0.264% (K1) & 0.151% (K2). Soil N levels at Matheran showed minimum variation; the lowest value was 0.171% (M1) & highest value was 0.263% (M3). At Nagla, soil N levels were 0.232% (N1) & 0.353% (N2). Soil of CEC showed 0.285% of N level.

4. Discussion

Moist soil is the key factor for mud-puddling. The soil moisture content at Yeoor, Karnala and CEC was found low as the mud-puddling spots were right next to seasonally fed streams. The streams in Karnala and Yeoor are monsoon fed and there is no addition of water other than groundwater, hence during post-monsoon months, these streams dry up. The soil moisture was higher in Nagla and Pelhar due to their geographical position. The Nagla block of Sanjay Gandhi National park is adjoining to Ulhas River Estuary therefore, it is under the influence of tidal fluctuations of saline estuarine water. The puddling spot has mixed vegetation, comprising of inland forest and mangrove forest. In Pelhar, the mud-puddling spot is at the backwaters of Pelhar Dam. The soil moisture content at Matheran showed variations because the puddling spots were at different locations but next to the perennial water source. One sampling station at Matheran showed higher moisture content because it was adjoining Charlotte Lake. Overall, in the present study moisture content was higher at Nagla, Matheran & Pelhar than that of Yeoor, Karnala and CEC.

Soil moisture content depends upon the type of soil. Soil texture of Yeoor, Karnala and CEC was Sandy Loam and Sandy Clay with a major fraction of sand in the soil. Sand cannot hold a good amount of water as it contains large size particles which have large spaces between them, thus, the water gets easily drained off. Therefore, soil moisture content was less at these three locations. Soil texture of Pelhar and Nagla is Loamy and Clayey, where, loamy soil is a combination of sand, silt and clay along with the presence of humus, due to which water stays in the soil for a longer period of time and clayey soil has good water holding capacity as they are made up of smallest particles. Soil texture of Matheran is Sandy Clay Loam, with a major fraction of sand present in the soil. Though sand does not have a good water holding capacity, moisture content of Matheran was pretty high as the sampling areas were closed to the perennial water outlets.

The Relative Humidity (RH) was found to be variable during the sampling period because it may depend on the time of the sampling. RH of Pelhar didn't show much variation since the sampling sites are located at the backwaters of Pelhar dam. If the water content is already high in the air, the evaporation of water from the soil will either happen at a very low rate or it won't take place at all. RH can be co-related with soil temperature gradient, For e.g. The highest RH calculated in the present study was 87% (Y5), Yeoor & the maximum temperature gradient calculated was 8°C (Y5), Yeoor, thus, it can be stated that increase in the temperature gradient gradually increases air moisture, while studying this co-relation all other factors need to be taken in consideration especially, climate, geographical area and soil moisture. In the present study, no insight to the co-relation between RH, soil moisture, air and soil temperature is given probably due to less number of samples.

The pH range observed during the sampling period was 6.5 to 7.7. The exact effect of pH on physio-chemical parameters of the soil needs to be investigated. United States Department of Agriculture, USDA stated that nitrogen cycle is inhibited by low & high soil pH levels; thus, stating that nitrogen levels are more between the pH range of 5.5 - 8.5. In the present study, no such estimation can be drawn. Lukac *et al*., (2011) [11] and Boczulak, S, (2013) [4] suggested the overall availability of Nitrogen in soil increases with increase in temperature. All the above-discussed factors need to be studied with respect to elevation, time & the microbial activity.

In the present study, Sodium (Na) & Nitrogen (N) was detected in the soil samples collected from puddling site. Both min. erals are limiting factors. The concentration of

sodium detected was very low than what was used as bait by Beck *et al.,* (1998) but was higher than concentrations used by Smedley and Eisner, (1996) [19]. While taking up Sodium from the soil, butterflies are known to excrete equal amount of Potassium (K^+) (Smedley and Eisner, 1996) [19]. This was further supported by Inoue *et. al.*, (2015) [10] who showed that butterflies who take moderate amount of Na^+ absorb Na^+ and excrete K^+ and butterflies who take high amount of Na^+ along with K^+ excrete excess Na^+ as well. Inoue *et. al*., (2015) [10] also demonstrated that K^+ contents are 8 to 9 times higher than Na^+ content in butterfly eggs, therefore female absorb all the K^+ taken up during puddling.

On 19/02/16, at Yeoor the puddling spot was dry & there was no puddling activity. We moistened the soil using normal, untreated tap water. As soon as, water started to evaporate, butterflies were attracted to this wet patch. This probably asserts that it is the soil which is a key factor in puddling rather than water. This is proven by the fact that some lycaenids & hesperids, use urine to moisten the soil or bird excreta. Thus, giving the fact that puddling is not dependent on the source of water. The only major role of water is to dissolve the soil nutrients and make them available to the butterflies in the aqueous form. Also it enlightens the fact given by Beck *et.al.,* (1999) [4] that butterflies of Family - Lycaenidae are attracted to the puddling site because of the olfactory cues, i.e., smell of the dissolved nutrients. This behavior can be correlated with wind. As wind carries smell along with it, this helps the butterflies to determine the puddling site. The attraction to the puddling site due to olfactory cues in Lycaenidae was more seen in the present study. Beck *et.al*., (1999) [4] stated that Lycaenids are small and cryptically closed wing colored butterflies, for them to detect their own family members puddling at the puddling site is very difficult as compared to the other butterflies who have conspicuously colored closed wings. this seems to be valid as we observed this during the study.

Mud-puddling behavior of butterflies is indeed a complex process which was studied extensively by several workers throughout the world. There is a high possibility of involvement of some environmental factors which must be regulating the complex process of mud puddling. This facet remains unexplored.

Thus, we propose a hypothesis - "mud puddling is the result of need of nutrients and moisture for survival and is regulated by various abiotic factors of the ecosystem. Most probably these factors are common throughout range of ecosystems."

5. Acknowledgement

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