Supplementary Material

Soil microplastic accumulation from plastic mulching in vegetable cropping systems across three agro-ecological regions in Nepal

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Statistical methods

The response variable was the number of microplastic particles counted in 50 gm of soil samples. The data were analyzed in R studio using negative binomial generalized linear models (NB GLMs) with a log link, fitted with the glm.nb function. This model was considered because microplastic count data are non- negative integers and variance greater than mean (Table S1). Model coefficients are expressed in log scale. The incidence rate ratio (IRR) indicates the relative change in expected microplastic count as compared with the reference group. The negative binomial dispersion parameter is represented by θ. The NB model uses a log link and a dispersion parameter (theta) that allows variance larger than the mean, which a Poisson model does not. The overall effect of each term was tested with Type II likelihood-ratio (LR) χ^2 tests. Estimated marginal means were obtained from the fitted models and back transformed to the original count scale. The pairwise comparisons were expressed as ratios of the estimated means. Tukey adjustment was applied when multiple comparison was conducted together. The significance level was 0.05. Forest soil samples were used only for the comparison between agriculture farms soil (mulched and non-mulched) and forest soil (Table S2 and S3). In all other analysis agriculture farm soils were only used.

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Table S1. Descriptive statistics of microplastic counts (particles per 50 g of soil) by group

Group	n	Mean	SD	Median	Min	Max
Land type (all soils)						
Farm	120	15.48	9.01	14.5	2	48
Forest	6	4.50	2.07	4.5	2	8
Mulching (farm soils)						
Non-mulched	60	13.7	7.42	13.5	2	36
Mulched	60	17.3	10.1	16	2	48
Depth (farm soils)						
0–10 cm	60	16.6	9.93	15	2	48
10–20 cm	60	14.4	7.94	14	2	34
Depth (forest soils)						
0–10 cm	3	5.33	2.52	5	3	8
10–20 cm	3	3.67	1.53	4	2	5
Mulching × depth (farm soils)						
Non-mulched, 0–10 cm	30	14.3	8.07	12.5	4	36
Non-mulched, 10–20 cm	30	13.0	6.78	14	2	25
Mulched, 0–10 cm	30	18.8	11.2	18.5	2	48
Mulched, 10–20 cm	30	15.8	8.85	15	2	34
District (farm soils)						
Kathmandu	40	20.3	8.75	19.5	6	45
Chitwan	40	16.6	7.73	16	8	48
Makwanpur	40	9.45	6.99	7.5	2	36
District × depth (farm soils)						
Kathmandu, 0–10 cm	20	22.2	9.32	22.5	10	45
Kathmandu, 10–20 cm	20	18.5	7.96	16	6	34
Chitwan, 0–10 cm	20	17.0	9.21	16.5	8	48
Chitwan, 10–20 cm	20	16.3	6.13	15	8	32
Makwanpur, 0–10 cm	20	10.5	7.91	8	2	36
Makwanpur, 10–20 cm	20	8.4	5.94	7	2	22

Note: Counts are particles per 50 g of soil. SD = standard deviation. Min and Max are the lowest and highest counts observed in the group.

Table S2. Negative binomial GLM comparing microplastic counts in agriculture farm and forest soils

Counts are non-negative integers whose variance exceeded the mean in the farm samples (Table S1). Negative binomial generalized linear model (log link) was used instead of a Poisson model. Land type (farm vs forest) was the only predictor and forest was the reference level. The likelihood-ratio (LR) test compares this model with an intercept-only model to test whether land type explains the counts.

Term	Estimate (log scale)	SE	z	P	IRR	95% CI of IRR
Intercept (forest)	1.5041	0.2844	5.288	1.24×10^{-7}	4.50	2.58–7.92
Farm	1.2351	0.2892	4.271	1.95×10^{-5}	3.44	1.94–6.06

Note: n = 126 samples (120 farm, 6 forest soil). IRR = incidence rate ratio (exp of the estimate); for the intercept it is the expected count in the reference group. Negative binomial dispersion parameter $\theta = 3.799$ (SE 0.597); the residual deviance was 129.69 (124 df), the null deviance was 146.46 (125 df), and the AIC was 875.09. LR test of land type against the intercept-only model: $\chi^2 = 15.92$, df = 1, $P = 6.60 \times 10^{-5}$.

Table S3. Wilcoxon rank-sum test comparing microplastic counts in farm and forest soils

A non-parametric check of Table S2 that makes no assumption about the count distribution. It was added because the groups are very unequal in size (120 farm vs 6 forest samples) so a test that does not rely on a fitted model gives a useful second opinion.

Comparison	n (farm)	n (forest)	Median (farm)	Median (forest)	W	P
Farm vs forest	120	6	14.5	4.5	58	0.000546

Note. Two-sided Wilcoxon rank-sum test with continuity correction (alternative hypothesis: true location shift is not equal to 0).

Table S4. Negative binomial GLM comparing microplastic counts in mulched and non-mulched farm soils

This test was performed within agriculture farm soils only (n = 120). An NB-GLM with Land use (mulched vs non-mulched soil samples) was done tests whether plastic mulching changes the expected count (non-mulched = reference). A Type II likelihood-ratio test gives the overall significance of Land use.

Term	Estimate (log scale)	SE	z	P	IRR	95% CI of IRR
Intercept (non-mulched)	2.61618	0.07418	35.266	$<2 \times 10^{-16}$	13.68	11.85–15.86

Term	Estimate (log scale)	SE	z	P	IRR	95% CI of IRR
Mulched	0.23260	0.10370	2.243	0.0249	1.26	1.03–1.55

*Note:*n =120 samples (60 mulched, 60 non-mulched). IRR = incidence rate ratio. Negative binomial dispersion parameter θ = 3.889 (SE 0.628); residual deviance 124.79 on 118 df; null deviance 129.82 on 119 df; AIC = 843.52. Type II LR test for mulching: $\chi^2 = 5.027$, df = 1, P = 0.02496.

Table S5. Negative binomial GLM comparing microplastic counts at 0–10 cm and 10–20 cm depth in farm soils

Only farm soils were included (n = 120), with depth as the predictor and 0–10 cm as the reference level. The LR test checks whether depth explains the counts. Forest depths are summarized descriptively only.

Term	Estimate (log scale)	SE	z	P	IRR	95% CI of IRR
Intercept (0–10 cm)	2.80639	0.07373	38.065	$<2 \times 10^{-16}$	16.55	14.35–19.17
Depth 10–20 cm	−0.13916	0.10498	−1.326	0.185	0.87	0.71–1.07

Note. IRR = incidence rate ratio. Negative binomial dispersion parameter θ = 3.764 (SE 0.602); residual deviance 124.84 on 118 df; null deviance 126.60 on 119 df; AIC = 846.71. Type II LR test for depth: $\chi^2 = 1.7565$, df = 1, P = 0.1851.

Table S6. Negative binomial GLM with mulching, depth and their interaction (coefficients).

The interaction model tests whether the effect of mulching differs between the two depths. Reference levels are non-mulched and 0–10 cm.

Term	Estimate (log scale)	SE	z	P
Intercept (non-mulched, 0–10 cm)	2.66259	0.10358	25.706	$<2 \times 10^{-16}$
Mulched	0.26949	0.14460	1.864	0.0624
Depth 10–20 cm	−0.09508	0.14727	−0.646	0.5185
Mulched × depth 10–20 cm	−0.07911	0.20588	−0.384	0.7008

Note. Negative binomial dispersion parameter θ = 3.967 (SE 0.644); residual deviance 124.78 on 116 df; null deviance 131.77 on 119 df; AIC = 845.66.

Table S7. Type II likelihood-ratio tests for the mulching × depth model

Term	LR χ^2	df	P
Mulching	5.0089	1	0.02522
Depth	1.7343	1	0.18786
Mulching × depth	0.1476	1	0.70081

Table S8. Estimated marginal means for mulching × depth (farm soils)

Model predicted mean counts for each mulching and depth combination, back-transformed from the log scale, shown with 95% confidence intervals. These are the values compared in Table S9.

Mulching	Depth	Mean count	SE	95% CI
Non-mulched	0–10 cm	14.3	1.48	11.7–17.6
Mulched	0–10 cm	18.8	1.89	15.4–22.9
Non-mulched	10–20 cm	13.0	1.36	10.6–16.0
Mulched	10–20 cm	15.8	1.62	12.9–19.3

Note. Counts are particles per 50 g of soil. Intervals are back-transformed from the log scale (confidence level 0.95).

Table S9. Pairwise comparisons of mulching × depth combinations (Tukey-adjusted)

Post-hoc comparison of all four mulching and depth combinations in Table S8, to show which specific groups differ. Tukey adjustment is used because six comparisons are made at once, which controls the overall false-positive rate.

Contrast	Ratio	SE	z	P (Tukey)
Non-mulched 0–10 cm / Mulched 0–10 cm	0.764	0.110	−1.864	0.2438
Non-mulched 0–10 cm / Non-mulched 10–20 cm	1.100	0.162	0.646	0.9171
Non-mulched 0–10 cm / Mulched 10–20 cm	0.909	0.133	−0.654	0.9142
Mulched 0–10 cm / Non-mulched 10–20 cm	1.440	0.209	2.507	0.0588
Mulched 0–10 cm / Mulched 10–20 cm	1.190	0.171	1.211	0.6200
Non-mulched 10–20 cm / Mulched 10–20 cm	0.827	0.121	−1.299	0.5635

Note. Ratios are ratios of estimated marginal means. Tests were performed on the log scale; P-value adjustment: Tukey method for comparing a family of 4 estimates.

Table S10. Negative binomial GLM comparing microplastic counts among the three districts (farm soils).

District (Kathmandu, Chitwan, and Makwanpur) was the predictor, with Kathmandu as the reference level. The LR test checks whether the districts differ overall.

Term	Estimate (log scale)	SE	z	P
Intercept (Kathmandu)	3.01185	0.07488	40.223	$<2 \times 10^{-16}$
Chitwan	−0.19944	0.10717	−1.861	0.0627
Makwanpur	−0.76584	0.11238	−6.815	9.45×10^{-12}

Note. Negative binomial dispersion parameter $\theta = 5.71$ (SE 1.03); residual deviance 123.82 on 117 df; null deviance 171.65 on 119 df; AIC = 810.07.

Type II LR test for district: $\chi^2 = 47.831$, df = 2, $P = 4.108 \times 10^{-11}$.

Table S11. Estimated marginal means by district (farm soils).

Model-predicted mean counts for each district from Table S10, back-transformed from the log scale, with 95% confidence intervals.

District	Mean count	SE	95% CI
Kathmandu	20.32	1.520	17.55–23.5
Chitwan	16.65	1.280	14.33–19.3
Makwanpur	9.45	0.792	8.02–11.1

Note. Counts are particles per 50 g of soil. Intervals are back-transformed from the log scale (confidence level 0.95).

Table S12. Pairwise comparisons among districts (Tukey-adjusted, farm soils).

Post-hoc comparison of the three districts, to show which districts differ after the overall district effect in Table S10. Tukey adjustment is used because three comparisons are made at once.

Contrast	Ratio	SE	z	P (Tukey)
Kathmandu / Chitwan	1.22	0.131	1.861	0.1502
Kathmandu / Makwanpur	2.15	0.242	6.815	<0.0001
Chitwan / Makwanpur	1.76	0.200	4.987	<0.0001

Note. Ratios are ratios of estimated marginal means. Tests were performed on the log scale; P-value adjustment: Tukey method for comparing a family of 3 estimates.

Table S13. Negative binomial GLM with district, depth and their interaction (coefficients).

The interaction model tests whether differences among districts depend on depth. Reference levels are Kathmandu and 0–10 cm.

Term	Estimate (log scale)	SE	z	P
Intercept (Kathmandu, 0–10 cm)	3.09784	0.10337	29.969	$<2 \times 10^{-16}$
Chitwan	−0.26462	0.14850	−1.782	0.0748
Makwanpur	−0.74646	0.15451	−4.831	1.36×10^{-6}
Depth 10–20 cm	−0.18007	0.14770	−1.219	0.2228
Chitwan × depth 10–20 cm	0.13802	0.21137	0.653	0.5138
Makwanpur × depth 10–20 cm	−0.04308	0.22223	−0.194	0.8463

Note. Negative binomial dispersion parameter $\theta = 5.93$ (SE 1.09); residual deviance 123.68 on 114 df; null deviance 176.19 on 119 df; AIC = 812.75.

Table S14. Type II likelihood-ratio tests for the district × depth model.

Test and rationale. Tests each term (district, depth, and their interaction) as a whole after accounting for the other terms in Table S13. The interaction row answers whether the district differences change with depth.

Term	LR χ^2	df	P
District	49.276	2	1.995×10^{-11}
Depth	2.629	1	0.1049
District × depth	0.741	2	0.6903

Table S15. Estimated marginal means for district × depth (farm soils).

Model-predicted mean counts for each district and depth combination from Table S13, back-transformed from the log scale, with 95% confidence intervals. These are the values compared in Tables S16 and S17.

District	Depth	Mean count	SE	95% CI
Kathmandu	0–10 cm	22.1	2.29	18.09–27.1
Chitwan	0–10 cm	17.0	1.81	13.79–21.0
Makwanpur	0–10 cm	10.5	1.21	8.38–13.2
Kathmandu	10–20 cm	18.5	1.95	15.04–22.7
Chitwan	10–20 cm	16.3	1.75	13.21–20.1
Makwanpur	10–20 cm	8.4	1.01	6.64–10.6

Note. Counts are particles per 50 g of soil. Intervals are back-transformed from the log scale (confidence level 0.95).

Table S16. Pairwise comparisons among districts within each depth (Tukey-adjusted).

It compares the districts separately at 0–10 cm and at 10–20 cm, to show whether the district pattern from Table S12 holds at both depths. Tukey adjustment is applied within each depth because three comparisons are made.

Contrast	Ratio	SE	z	P (Tukey)
Depth 0–10 cm				
Kathmandu / Chitwan	1.30	0.193	1.782	0.1756
Kathmandu / Makwanpur	2.11	0.326	4.831	<0.0001
Chitwan / Makwanpur	1.62	0.254	3.075	0.0060
Depth 10–20 cm				
Kathmandu / Chitwan	1.13	0.171	0.842	0.6770
Kathmandu / Makwanpur	2.20	0.352	4.943	<0.0001
Chitwan / Makwanpur	1.94	0.312	4.121	0.0001

Note. Ratios are ratios of estimated marginal means. Tests were performed on the log scale; P-value adjustment: Tukey method for comparing a family of 3 estimates.

Table S17. Comparison of depths within each district.

Simple contrasts that compare 0–10 cm with 10–20 cm separately in each district, to see whether depth matters in any one district.

Contrast (0–10 cm / 10–20 cm)	Ratio	SE	z	P
Kathmandu	1.20	0.177	1.219	0.2228
Chitwan	1.04	0.158	0.278	0.7809
Makwanpur	1.25	0.208	1.344	0.1790

Note. Ratios are ratios of estimated marginal means. Tests were performed on the log scale.

Table S18. Negative binomial GLM with district, mulching, depth and all interactions (coefficients).

This full model includes district, mulching and depth together with all two- and three-way interactions. It checks whether the main findings hold when all three factors are considered at once. Reference levels are Kathmandu, non-mulched and 0–10 cm.

Term	Estimate (log scale)	SE	z	P
Intercept	2.88480	0.14051	20.531	$<2 \times 10^{-16}$
Chitwan	−0.24574	0.20258	−1.213	0.2251
Makwanpur	−0.47786	0.20714	−2.307	0.0211
Mulched	0.38856	0.19413	2.002	0.0453
Depth 10–20 cm	−0.22454	0.20221	−1.110	0.2668
Chitwan × mulched	−0.03189	0.27953	−0.114	0.9092
Makwanpur × mulched	−0.50297	0.29173	−1.724	0.0847
Chitwan × depth 10–20 cm	0.44195	0.28652	1.543	0.1229
Makwanpur × depth 10–20 cm	−0.18092	0.30286	−0.597	0.5503
Mulched × depth 10–20 cm	0.07354	0.27820	0.264	0.7915
Chitwan × mulched × depth 10–20 cm	−0.56539	0.39810	−1.420	0.1555
Makwanpur × mulched × depth 10–20 cm	0.28010	0.42104	0.665	0.5059

Note. Negative binomial dispersion parameter $\theta = 7.06$ (SE 1.38); residual deviance 124.4 on 108 df; null deviance 197.9 on 119 df; AIC = 811.31.

Table S19. Type II likelihood-ratio tests for the full three-way model
Tests each main effect and interaction in Table S18 as a whole.

Term	LR χ^2	df	P
District	53.584	2	2.314×10^{-12}
Mulching	6.195	1	0.01281
Depth	2.916	1	0.08768
District × mulching	3.839	2	0.14667
District × depth	0.967	2	0.61655
Mulching × depth	0.070	1	0.79163
District × mulching × depth	4.236	2	0.12026

Table S20. Correlation analysis between microplastic and soil pH and with soil organic matter

Variable	Correlation
Soil_pH	0.33206
Soil Om	0.048703

Table S21. NB GLM Soil organic matter only

	Estimate	SE	z_value	p_value	IRR
(Intercept)	2.670173	0.138311	19.30561	4.82E-83	14.44247
SoilOm	0.030436	0.056549	0.53822	0.590425	1.030904

Interpretation:

Coefficient ≈ 0.03, p ≈ 0.59 → not significant

- No evidence that OM affects microplastic counts
- Line in scatterplot is flat

Table S22. NB GLM Soil pH only

	Estimate	SE	z_value	p_value	IRR
(Intercept)	1.403578	0.340155	4.126285	3.69E-05	4.069735
SoilpH	0.207854	0.052776	3.938382	8.20E-05	1.231033

Interpretation:

Coefficient for SoilpH = 0.208, p < 0.001 → significant positive effect

Each unit increase in pH increases expected microplastic count by ~22% (IRR = exp (0.208) ≈ 1.23)

Table S23. NB GLM (Soil pH + Soil organic matter): Independent effect

	Estimate	SE	z value	p value	IRR
(Intercept)	1.418706	0.340034	4.172245	3.02E-05	4.131769
SoilpH	0.216763	0.055525	3.903857	9.47E-05	1.242049
SoilOm	-0.03191	0.056218	-0.56767	0.57026	0.968591

- Both predictors included simultaneously, to see independent effects
- Soil pH: still significant
- Soil OM: still non-significant

Table S24. Number of years of continuous plastic mulching in mulched fields

Sample Id	Makwanpur (years)	Chitwan (years)	Kathmandu (years)
Farm 1	2	9	5
Farm 2	5	8	5
Farm 3	2	7	6
Farm 4	2	5	7
Farm 5	2	5	5
Farm 6	2	5	6
Farm 7	5	2	4
Farm 8	3	4	4
Farm 9	2	8	12
Farm 10	5	3	8

Table 25. Techniques of waste disposals

Mulched field	Method of waste disposal	Non-mulched field	Method of waste disposal
Makwanpur	Municipal waste collection	Makwanpur	Municipal waste collection
Chitwan	Burning the plastic in the field	Chitwan	Burning and municipal waste collection
Kathmandu	Burning/ dumping in the field and municipal waste collection	Kathmandu	Burning/ dumping in the field

Table S26. Details of sampling points of the study area

Sample Id	District	Latitude	Longitude	Land use type
Chitwan forest	Chitwan	27.68362	84.45913	Forest soil
CMF1	Chitwan	27.67955	84.49352	Mulched soil
CMF10	Chitwan	27.67871	84.50197	Mulched soil
CMF2	Chitwan	27.68021	84.49442	Mulched soil
CMF3	Chitwan	27.6774	84.49545	Mulched soil
CMF4	Chitwan	27.67554	84.49596	Mulched soil
CMF5	Chitwan	27.6733	84.49532	Mulched soil
CMF6	Chitwan	27.67318	84.49957	Mulched soil
CMF7	Chitwan	27.67295	84.49855	Mulched soil
CMF8	Chitwan	27.67312	84.49495	Mulched soil
CMF9	Chitwan	27.67789	84.50144	Mulched soil
CNF1	Chitwan	27.68894	84.4503	Non- mulched soil
CNF10	Chitwan	27.68825	84.44141	Non- mulched soil
CNF2	Chitwan	27.68932	84.45188	Non- mulched soil
CNF3	Chitwan	27.68919	84.45307	Non- mulched soil
CNF4	Chitwan	27.67601	84.35058	Non- mulched soil
CNF5	Chitwan	27.68175	84.44127	Non- mulched soil
CNF6	Chitwan	27.68419	84.44092	Non- mulched soil
CNF7	Chitwan	27.68455	84.44267	Non- mulched soil
CNF9	Chitwan	27.68491	84.44092	Non- mulched soil
CNF9	Chitwan	27.68467	84.44172	Non- mulched soil
Kathmandu forest	Kathmandu	27.6668	85.24953	Forest soil
KMF1	Kathmandu	27.76927	85.29784	Mulched soil
KMF10	Kathmandu	27.66407	85.26204	Mulched soil
KMF2	Kathmandu	27.76961	85.29591	Mulched soil
KMF3	Kathmandu	27.77214	85.29667	Mulched soil
KMF4	Kathmandu	27.77763	85.29024	Mulched soil
KMF5	Kathmandu	27.76804	85.30416	Mulched soil
KMF6	Kathmandu	27.78107	85.30319	Mulched soil
KMF7	Kathmandu	27.79258	85.32811	Mulched soil
KMF8	Kathmandu	27.79099	85.32775	Mulched soil
KMF9	Kathmandu	27.76691	85.30736	Mulched soil
KNF1	Kathmandu	27.78204	85.29158	Non- mulched soil
KNF10	Kathmandu	27.66637	85.25157	Non- mulched soil
KNF2	Kathmandu	27.78167	85.29008	Non- mulched soil
KNF3	Kathmandu	27.77978	85.28987	Non- mulched soil
KNF4	Kathmandu	27.76733	85.30813	Non- mulched soil
KNF5	Kathmandu	27.77962	85.31357	Non- mulched soil
KNF6	Kathmandu	27.77895	85.31117	Non- mulched soil
KNF7	Kathmandu	27.794	85.3239	Non- mulched soil
KNF8	Kathmandu	27.74718	85.30512	Non- mulched soil
KNF9	Kathmandu	27.66211	85.26011	Non- mulched soil
Makwanpur forest	Makwanpur	27.6437	85.1081	Forest soil
MMF1	Makwanpur	27.65025	85.10829	Mulched soil

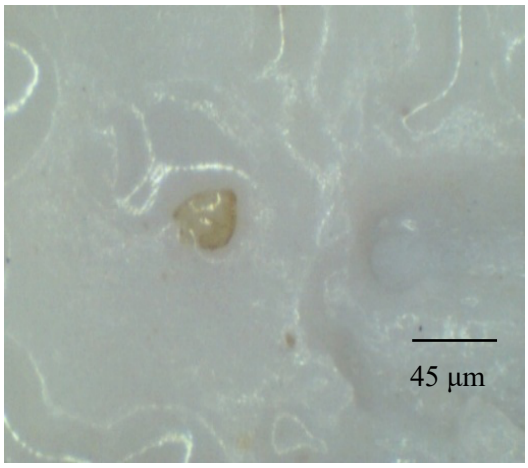
MMF10	Makwanpur	27.64134	85.11925	Mulched soil
MMF2	Makwanpur	27.64756	85.12412	Mulched soil
MMF3	Makwanpur	27.65206	85.12365	Mulched soil
MMF4	Makwanpur	27.65284	85.12476	Mulched soil
MMF5	Makwanpur	27.6533	85.12531	Mulched soil
MMF6	Makwanpur	27.65291	85.12568	Mulched soil
MMF7	Makwanpur	27.65425	85.12684	Mulched soil
MMF8	Makwanpur	27.65488	85.12601	Mulched soil
MMF9	Makwanpur	27.65566	85.12535	Mulched soil
MNF1	Makwanpur	27.65364	85.10438	Non- mulched soil
MNF10	Makwanpur	27.63277	85.09106	Non- mulched soil
MNF2	Makwanpur	27.65375	85.1061	Non- mulched soil
MNF3	Makwanpur	27.64732	85.12242	Non- mulched soil
MNF4	Makwanpur	27.64762	85.12422	Non- mulched soil
MNF5	Makwanpur	27.65253	85.12439	Non- mulched soil
MNF6	Makwanpur	27.65425	85.1252	Non- mulched soil
MNF7	Makwanpur	27.64131	85.11931	Non- mulched soil
MNF8	Makwanpur	27.64219	85.11217	Non- mulched soil
MNF9	Makwanpur	27.63988	85.11309	Non- mulched soil

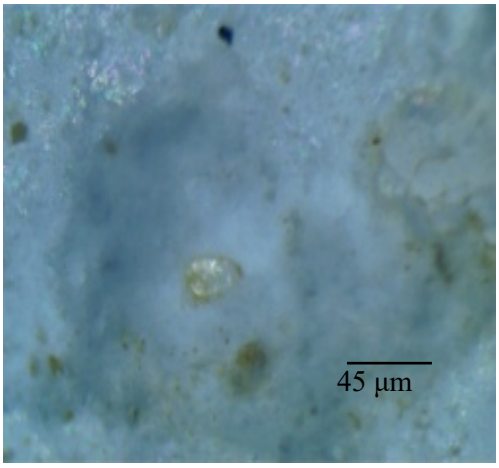
Table S27. Meteorological and soil environmental conditions of different agro ecological regions (soil sampling site).

Location	Mean temperature (°C)	Mean humidity (%)	Total precipitation (mm)	Mean precipitation (mm)	Mean soil temperature (°C)	Mean soil moisture (°C)	Mean wind speed (ms^{-})
Chitwan	23.52234	71.23998	1178.41	0.133789	23.59821	0.271086	4.786598
Kathmandu	17.92208	71.45907	2170.624	0.246438	18.44376	0.29091	5.568562
Makwanpur	15.7082	75.17018	1761.724	0.200014	16.72719	0.296973	4.158864

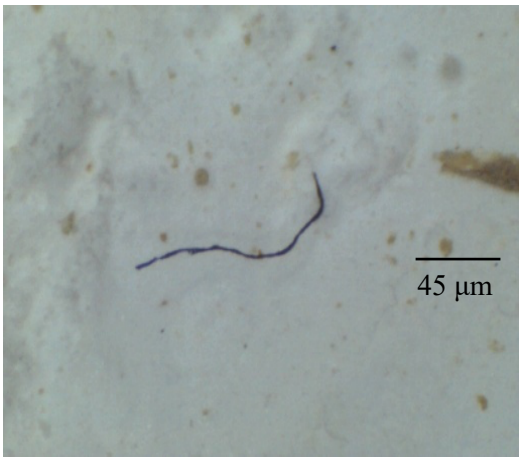
Figure S1: Shapes of microplastics a) Film b) Fiber c) Fragment

a





b





c



