Development of a pre-emergent nanoherbicide: from efficiency evaluation to assessment of environmental fate and risks to soil microorganisms.

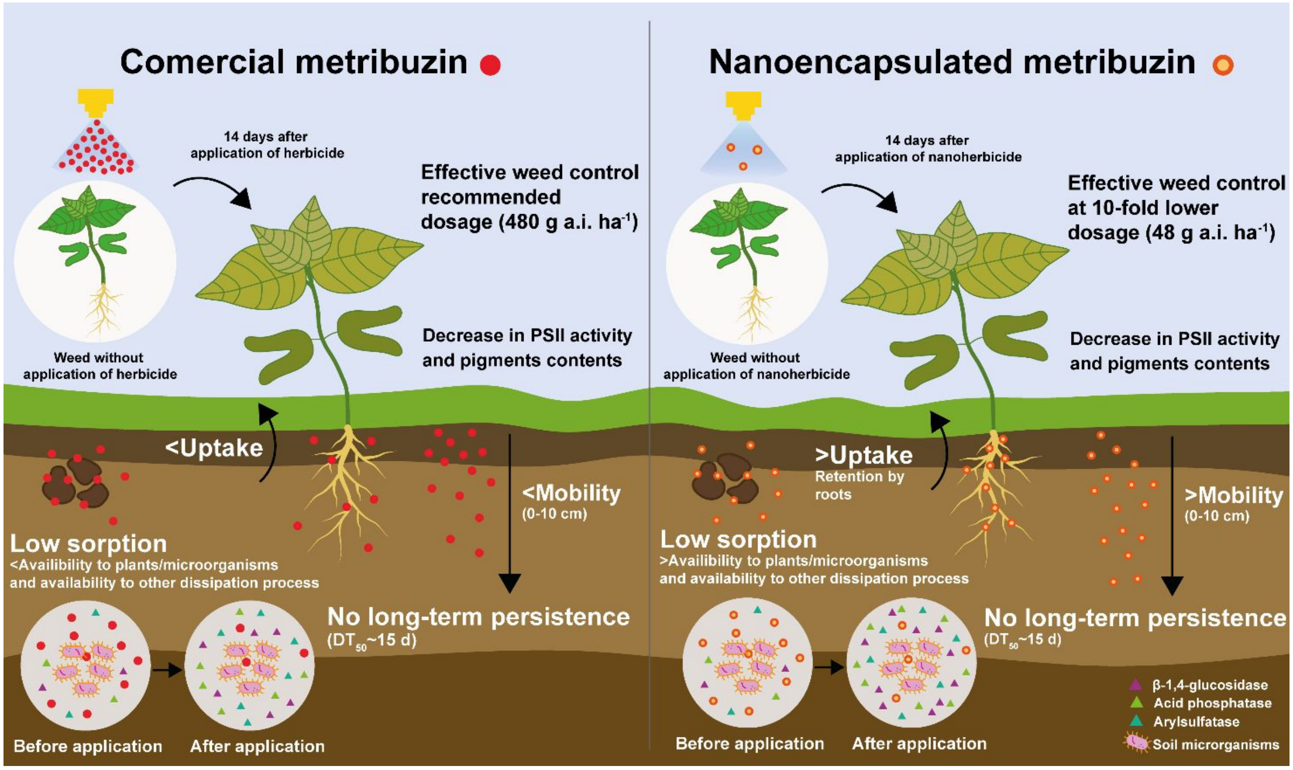
Vanessa Takeshita[1]*, Lucas Bragança Carvalho[2], Juliana Aparecida Galhardi[2], Gustavo Vinícios Munhoz-Garcia[1], Rodrigo Floriano Pimpinato[1], Halley Caixeta Oliveira[3], Valdemar Luiz Tornisielo[1], Leonardo Fernandes Fraceto[3]*.

[1]Center of Nuclear Energy in Agriculture, University of São Paulo, Av. Centenário 303, 13400-970, Piracicaba, SP, Brazil; [2]Institute of Science and Technology, São Paulo State University (UNESP), Av. Três de Março 511, 18087-180, Sorocaba, SP, Brazil; [3]Department of Animal and Plant Biology, State University of Londrina, PR 445, km 380, 86057-970, Londrina, PR, Brazil.

Supporting Information Placeholder

ABSTRACT: Nanoparticles based on biodegradable polymers have been shown to be excellent herbicide carriers, improving weed control and protecting the active ingredient in the crop fields. Metribuzin is often found in natural waters, which raises environmental concerns. Nanoencapsulation of this herbicide could be an alternative to reduce its losses to the environment and improve gains in its efficiency. However, there is a paucity of information about the behavior of nanoformulations of herbicides in environmental matrices. In this study, the stability of nanoencapsulated metribuzin in polymeric nanoparticles (*nano*MTZ) was verified over time, as well as its dissipation in different soils, followed by the effects on soil enzymatic activity. Physiological parameters and control effects of *nano*MTZ on *Ipomoea grandifolia* plants were investigated. No differences were verified in the half-life of nanoencapsulated metribuzin when compared to a commercial formulation of the herbicide. Moreover, no suppressive effects on soil enzymatic activities were observed. The retention of *nano*MTZ in the tested soils was lower when compared to its commercial analogue. However, mobility of nanoencapsulated metribuzin was not greatly increased, reflecting a low risk of groundwater contamination. Weed control was effective even at the lowest dose of *nano*MTZ (48 g a.i. ha^{-1}), which was consistent with higher efficiency of *nano*MTZ compared to the conventional herbicide in inhibiting PSII activity and decreasing pigment levels. Overall, we verified that *nano*MTZ presented a low environmental risk, with increased weed control.

Keywords: nanopesticide, sorption-desorption, leaching, biodegradation, chemical control, soil enzyme activity.



ABSTRACT GRAPHIC

SUPPLEMENTARY MATERIAL

A. Calibration of the soil enzymatic activity assays

Enzyme activities were determined after 24 h of incubation at 30 °C in the dark, under constant agitation. For the soil enzyme activity measurements, the substrate fluorescence intensities were analyzed using an excitation wavelength of 330 nm, with fluorescent emission measurements at 460 nm using a microplate reader (Tecan, Infinite M200). A calibration curve using increasing concentrations of Mub (0.1 - 10 mM) was used to calculate the enzymatic activities (equation: fluorescence intensity = Mub (mM) × 4272 + 485; R^2 = 0.999; limit of detection = 0.07 mM of Mub for acid phosphatase, 0.02 mM of Mub for arylsulfatase, and 0.04 mM of Mub for β-1,4-glucosidase; limit of quantification = 0.22 mM of Mub for acid phosphatase, 0.07 mM of Mub for arylsulfatase, and 0.11 mM of Mub for β-1,4-glucosidase). Enzyme activities were expressed as nmol Mub g^{-1} h^{-1}, normalized for control samples, i.e., no treatment added to the soils.

Tabel S1. Encapsulation efficiency (EE) of nanoMTZ over 120 days after preparation of the formulation.

Time (days)	Encapsulation efficiency (%)[a]
0	75.02 ± 0.05
15	74.36 ± 0.10
30	74.52 ± 3.01
45	75.00 ± 0.04
60	74.00 ± 0.27
90	73.99 ± 0.15
120	76.75 ± 0.05

[a]Standard error of the mean of triplicates (*n* = 3).

Table S2. Physical-chemical properties of soils.

Parameters[a]	Soil[b]		
	Clay	Sandy loam - 1 (SL-1)	Sandy loam - 2 (SL-2)
Total sandy ($g.kg^{-1}$)	368	710	780
Silt($g.kg^{-1}$)	227	64	44
Clay ($g.kg^{-1}$)	405	226	176
pH ($CaCl_2$)	5.2	4.6	4
O.M. ($g.dm^{-3}$)	47	9	17
O.C. (%)	2.73	0.52	0.99
P ($mg.dm^{-3}$)	51	36	17
K ($mmolc.dm^{-3}$)	3.3	1.3	3
Ca ($mmolc.dm^{-3}$)	40	15	15
Mg ($mmolc.dm^{-3}$)	16	12	8
H+Al ($mmolc.dm^{-3}$)	13	12	22
SB ($mmolc.dm^{-3}$)	59.3	28.3	26
CEC ($mmolc.dm^{-3}$)	72.3	40.3	48
V (%)	82	70	54

[a]Soils analyzed at the Laboratory of Mineral Fertilizers of the Superior School of Agriculture "Luiz de Queiroz", University of São Paulo, Piracicaba, São Paulo, Brazil.
[b]Soil classification according to the Brazilian Soil Classification System (Embrapa, 2018). Eutroferric Red Nitosol (NITOSSOLO VERMELHO Eutroférrico típico - Nvef) (Clay), Dystrophic Red-Yellow Argisol (ARGISSOLO VERMELHO-AMARELO Distrófico – PVAd) (Sandy loam - 1), Dystrophic Red-Yellow Latosol (LATOSSOLO VERMELHO-AMARELO – LVAd) (Sandy loam - 2).

Table S3. Bounded residue (no extracted ^{14}C-metribuzin) and total of $^{14}CO_2$ accumulated in 28 days after the pesticide's application (DAA). Different lowercase letters indicate significant differences between formulations within the same soil, while different uppercase letters indicate significant differences between soils for the same formulation, by Tukey's test ($p<0.05$), for each incubation time.

Soil	Time (DAA)	Bounded residue		$^{14}CO_2$ accumulated	
		*nano*MTZ (%)	MTZ (%)	*nano*MTZ (%)[a]	MTZ (%)
Clay	0	9.03 ± 0.26[a] aA	9.33 ± 0.49 aA	0 aA	0 bA
	7	30.16 ± 0.20 aA	30.35 ± 0.29 aA	1.97 ± 0.11 aC	2.52 ± 0.122 aB
	14	42.66 ±1.00 aB	39.62 ± 0.79 aB	4.73 ± 0.26 aC	5.69 ± 0.19 bB
	21	48.48 ± 0.93 aB	42.85 ± 0.54 aB	5.86 ± 0.32 aC	10.11 ± 0.57 bB

	28	54.44 ± 0.10 aB	54.64 ± 1.10 aB	6.98 ± 0.48 aC	11.83 ± 0.72 bB
SL-1	0	7.64 ± 0.28 aB	7.94 ± 0.25 aB	0 aB	0 aB
	7	51.31 ± 2.02 aB	47.63 ± 1.66 aB	3.19 ± 0.13 aA	3.66 ± 0.52 aA
	14	60.94 ± 0.13 aA	58.60 ± 1.40 aA	9.89 ± 0.25 aA	10.22 ± 0.62 aA
	21	60.40 ± 1.77 aA	51.73 ± 0.59 bAB	15.02 ± 0.20 aA	15.09 ± 0.70 aA
	28	69.25 ± 0.50 aA	57.86 ± 1.01 bAB	19.20 ± 0.12 aA	19.20 ± 0.51 aA
SL-2	0	5.73 ± 0.01 aC	5.46 ± 0.27 aC	0 aB	0 aA
	7	47.78 ± 1.80 aB	46.95 ± 2.92 aB	3.03 ± 0.05 aB	2.51 ± 0.01 bA
	14	56.62 ± 1.04 aA	60.92 ± 2.37 aA	7.87 ± 0.13 aB	6.46 ± 0.20 aB
	21	63.38 ± 1.00 aA	58.27 ± 0.58 bA	11.49 ± 0.20 aB	10.13 ± 0.22 aB
	28	66.70 ± 0.87 aA	59.43 ± 0.92 bA	14.86 ± 0.06 aB	13.54 ± 0.20 aB

[a]Standard error of the mean of duplicates ($n = 2$). Percentage in relation of total ^{14}C-metribuzin applied.

Table S4. Concentration of metribuzin extracted from enzymatic activity assay, after 2, 7, 14, 21 and 28 days after the pesticide's application (DAA). Lowercase letters indicate the differences between the formulations within each soil and capital letters indicate the differences between each soil within each formulation, by Tukey's test ($p<0.05$).

Formulation	Soil	Concentration of metribuzin (mg kg^{-1})				
		24 h[ns]	7 DAA[ns]	14 DAA	21 DAA	28 DAA
*nano*MTZ	Clay	0.92 ± 0.09	0.71 ± 0.33	0.57 ± 0.20 aA	0.15 ± 0.06 aB	0.10 ± 0.02 aA
*nano*MTZ	SL-1	0.65 ± 0.38	0.43 ± 0.23	0.32 ± 0.17 aA	0.12 ± 0.06 aA	0.06 ± 0.03 aA
*nano*MTZ	SL-2	0.46 ± 0.25	0.27 ± 0.24	0.04 ± 0.04 aC	0.02 ± 0.01 aC	0.004 ± 0.004 aB
MTZ	Clay	0.69 ± 0.40	0.52 ± 0.37	0.19 ± 0.18 bA	0.06 ± 0.01 aA	0.03 ± 0.02 bA
MTZ	SL-1	0.42 ± 0.32	0.33 ± 0.23	0.04 ± 0.02 bA	0.01 ± 0.01 bB	0.01 ± 0.01 bA
MTZ	SL-2	0.47 ± 0.19	0.15 ± 0.08	0.01 ± 0.01 aA	0.02 ± 0.01 aB	0.002 ± 0.002 aA

[ns]No significant differences were found (F<0,05).

Table S4. Percentage of sorption ^{14}C-metribuzin in first step of sorption studies. Determination of soil:solution ratio (v/v) (>20% of sorption or values between 50-80%) according OECD guideline testing for chemical (2000).

Soil	Soil:solution ratio	*nano*MTZ (%)	MTZ (%)
Clay	01:01	44,89 ± 0,63[a]	58,33 ± 0,67
	01:02	16,02 ± 1,73	51,56 ± 0,53
	01:05	21,27 ± 1,54	18,46 ± 0,88
SL-1	01:01	50,29 ± 0,16	62,83 ± 0,60
	01:02	25,54 ± 2,21	61,78 ± 1,03
	01:05	27,97 ± 1,83	31,58 ± 1,44
SL-2	01:01	30,96 ± 1,15	50,57 ± 2,06
	01:02	3,90 ± 1,81	46,96 ± 0,63
	01:05	24,07 ± 7,06	16,08 ± 0,91

[a]Standard error of the mean of duplicates ($n = 2$).

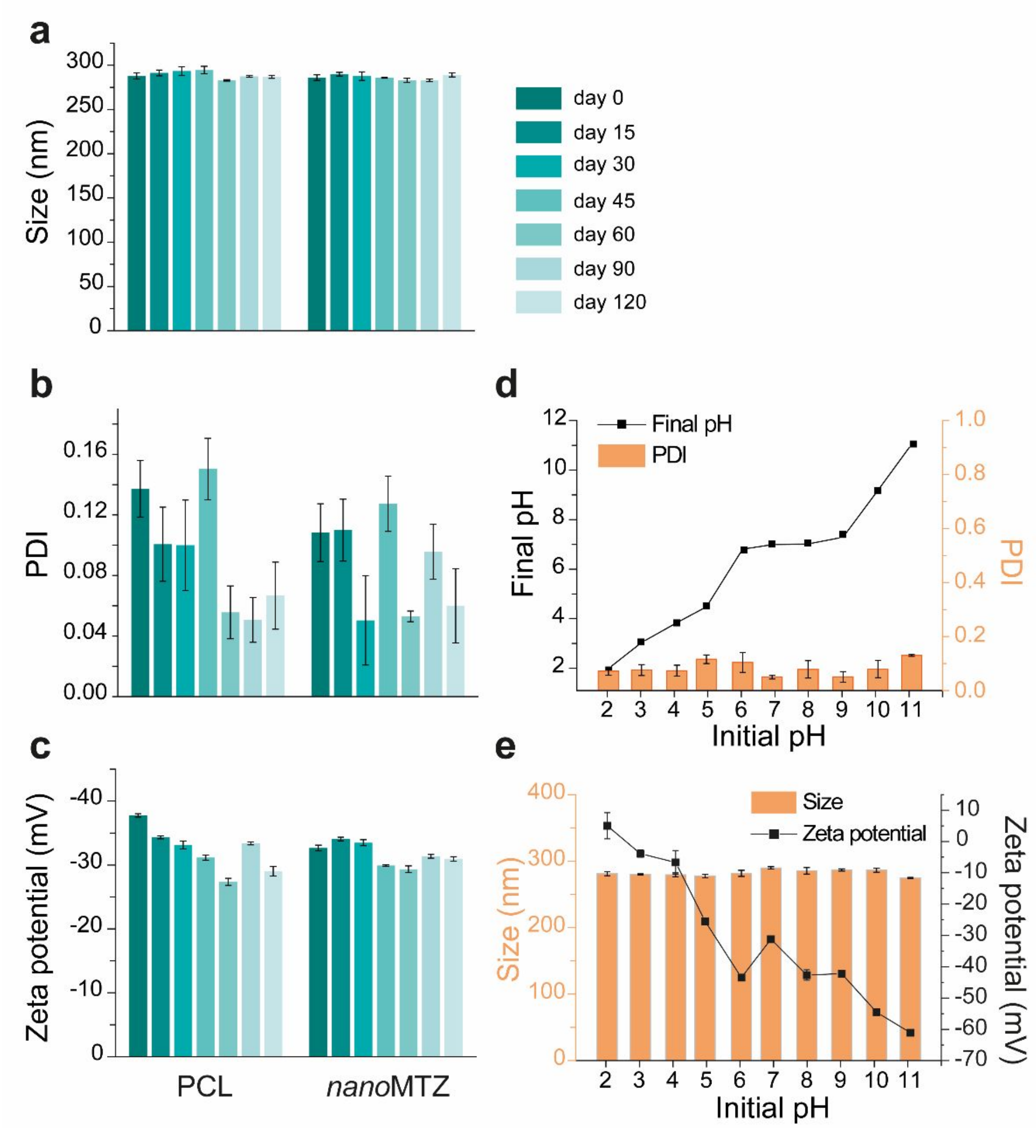


Figure S1. Characterization and stability of the *nano*MTZ formulation over 120 days after nanoencapsulation. Average size (a), polydispersion index (b) and zeta potential (c), obtained using the DLS technique. Variation of the polydispersity index (IPD) (d), size and zeta potential (e) of PCL nanoparticles containing metribuzin, as a function of pH (2-11).

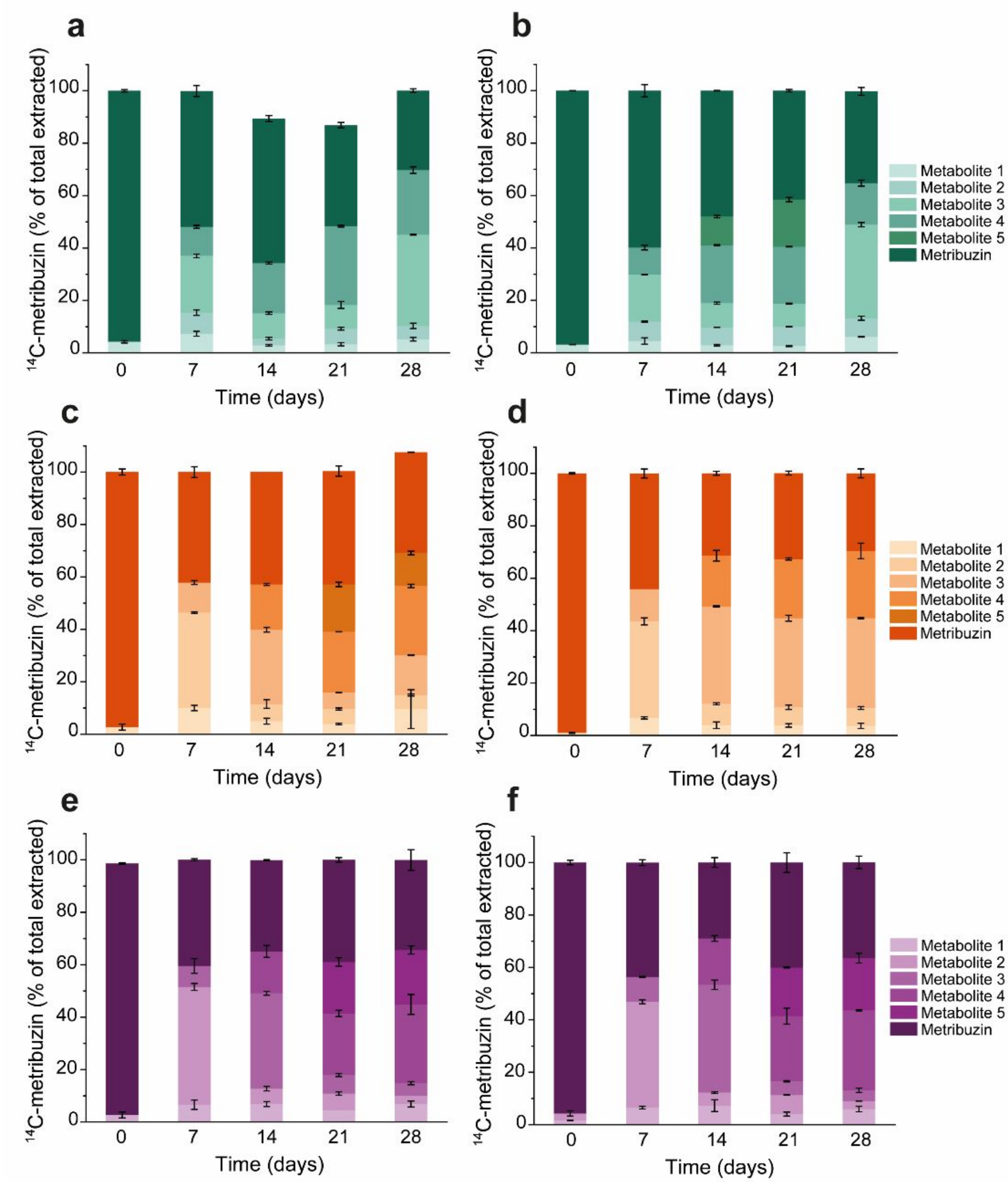


Figure S2. Parental ^{14}C-metribuzin and metabolites formed over 28 days after application of *nano*MTZ and MTZ formulations in soils (Clay (a and b), SL-1 (b and c) e, SL-2 (d and f), respectively). Bars indicate the mean ± the standard error of the mean (n = 2).

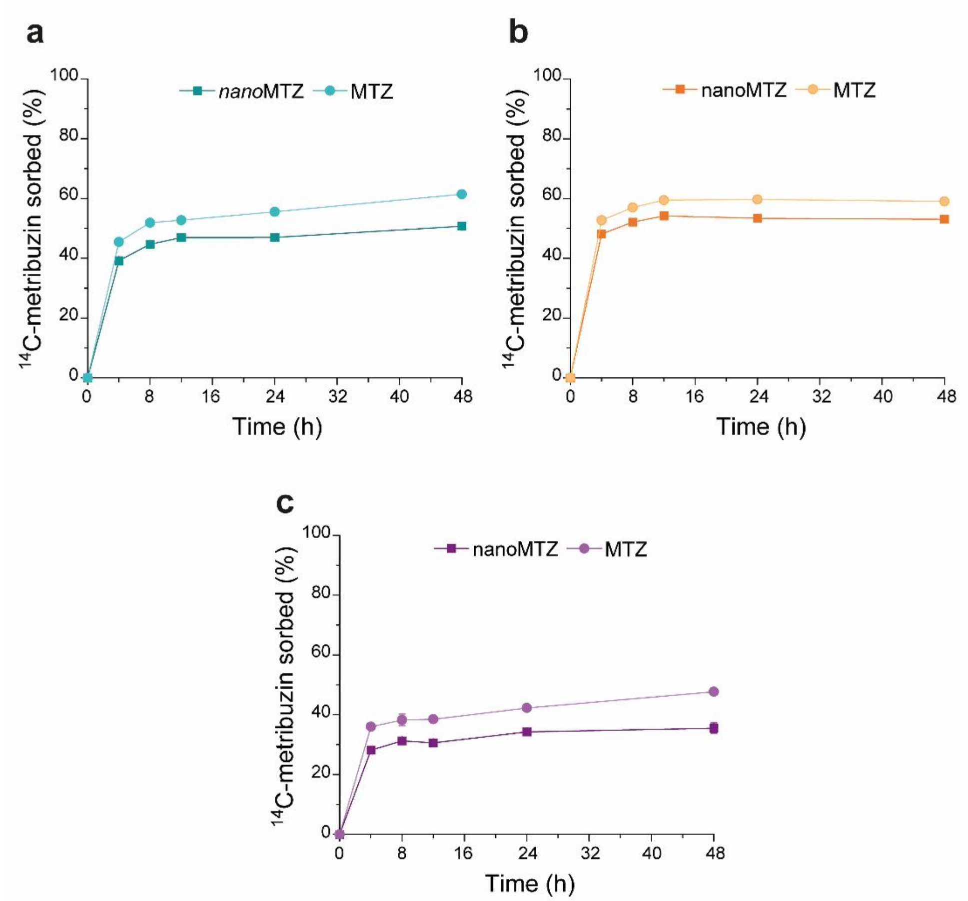


Figure S3. Sorption kinetics of the formulations followed for 48 hours after application on Clay (a), SL-1 (b) and SL-2 (c). Points represent mean sorption ± standard error of the mean (n = 2).

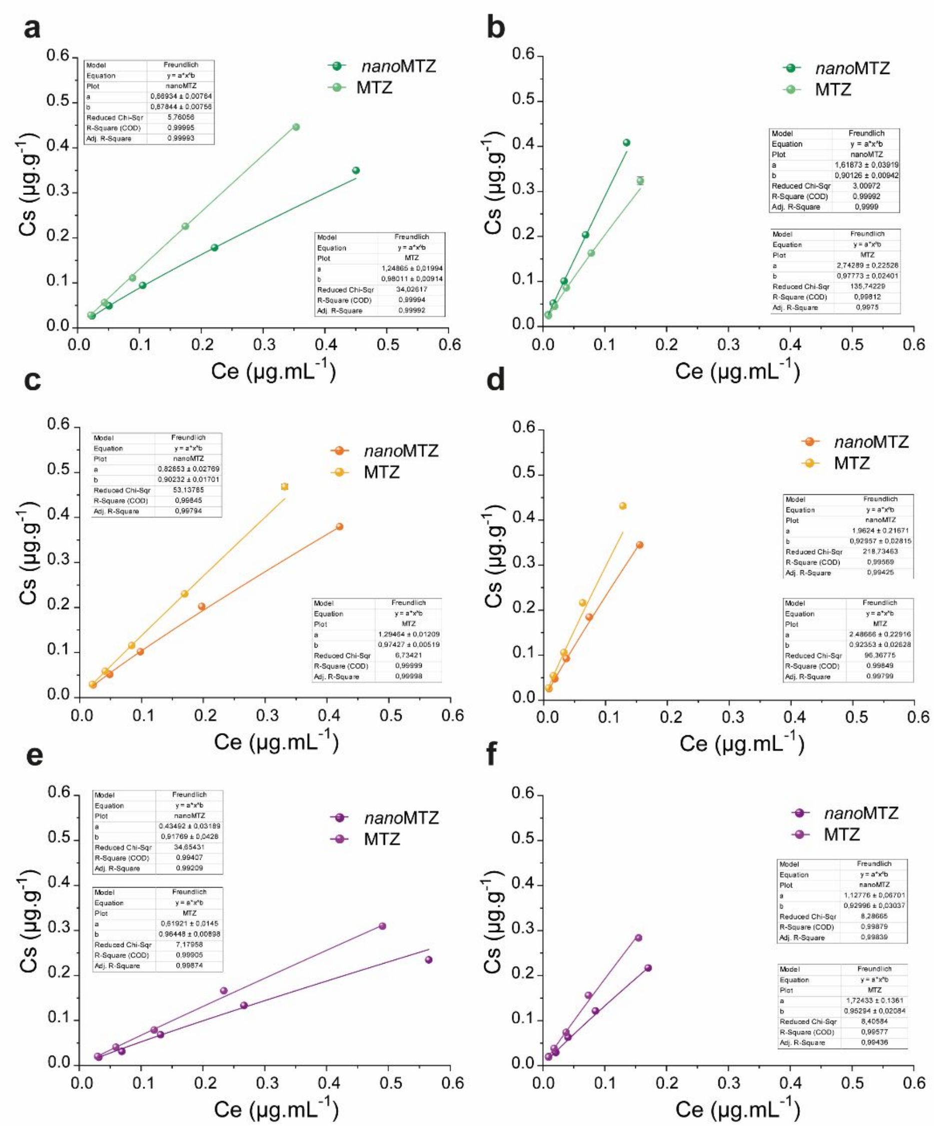


Figure S4. Sorption (a, c, d) and desorption (b, d, f) isotherms for Clay, SL-1 and SL-2, respectively. The points represent the mean of the sorption coefficient ± standard error of the mean ($n = 2$).

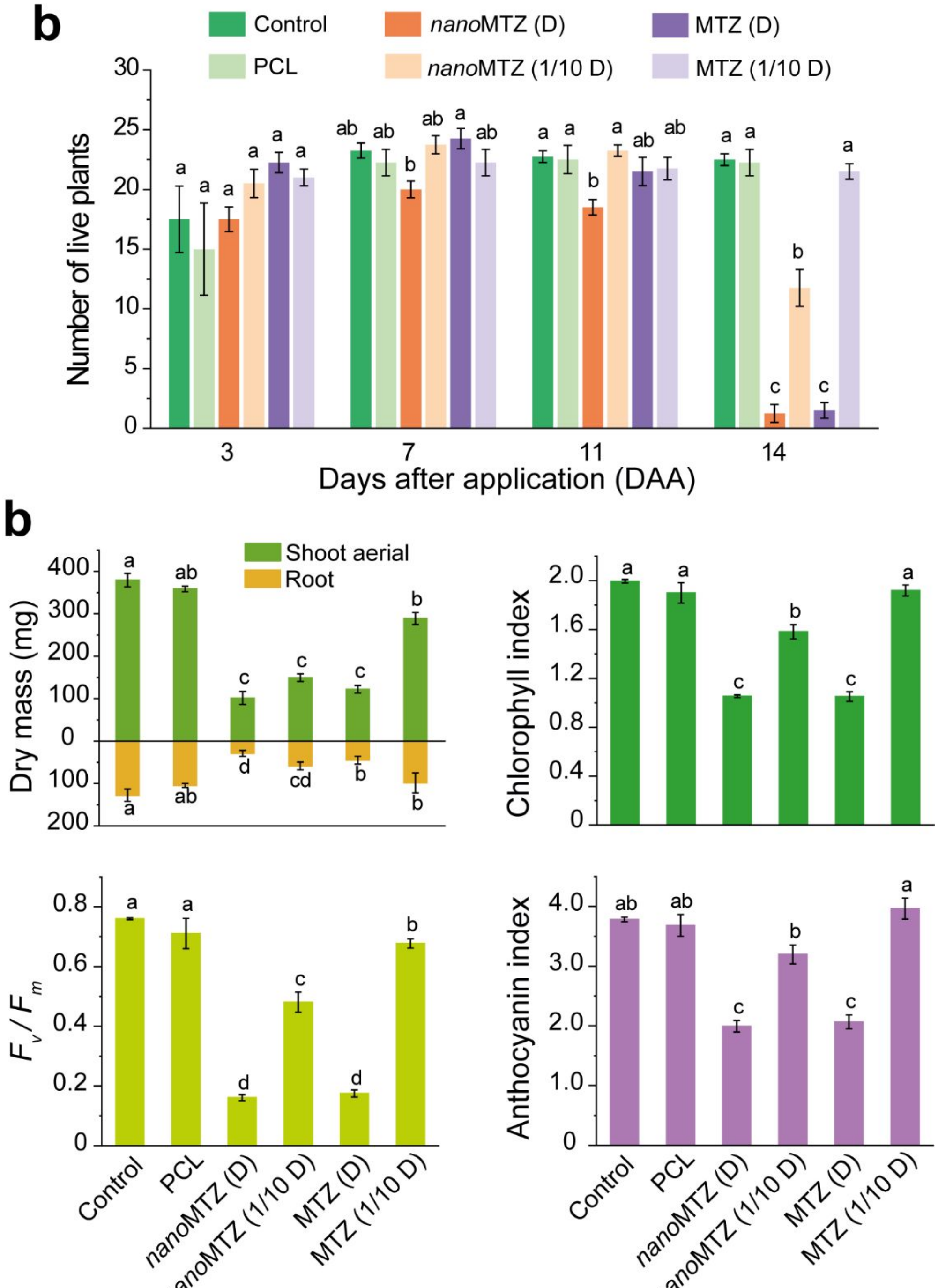


Figure S5. Number of live plants (a), and physiological parameters (b). Bars represent the mean and standard error of the mean (n = 4). Different lowercase letters indicate differences between treatments on each days after application (DAA) for the number of plants (a), shoot and root dry mass at 14 DAA (b), F_v/F_m (maximum quantum efficiency of PSII), chlorophyll *a* index and anthocyanin index at 7 DAA (b), by Tukey test (p<0.05).