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Specific biotests to assess eco-toxicity of biodegradable polymer materials in soil

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ABSTRACT

Eco-toxicity investigation of polymer materials was considered extremely necessary for their potential menace, which was widely use as mulching materials in agricultural. In this study, polyethylene (PE), polystyrene (PS) and synthetic biomaterials-Ecoflex and cellulose were applying into soil cultivated with two potential indicator plants species: oat (*Avena sativa*) and red radish (*Raphanus sativum*). Variety of chemical, biochemical parameters and enzyme activity in soil were proved as effective approach to evaluate polymers phytotoxicity in plant-soil mesocosm. The F-value of biomass, pH, heavy metal and electoral conductivity of *Raphanus* behaved significant different from T0. Significant analysis results indicated biodegradation was fast in PE than PS, besides, heavy metals were dramatically decrease in the end implied the plant absorption may help decrease heavy metal toxicity. The increase value at T2 of Dehydrogenase activity (0.84 higher than average value for *Avena* & 0.91 higher for *Raphanus*), Metabolic Index (3.12 higher than average value for *Avena* & 3.81 higher for *Raphanus*) means during soil enzyme activity was promoted by biodegradation for its heterotrophic organisms' energy transportation was stimulated. Statistics analysis was carried on Biplot PC1 (24.2% of the total variance), PC2 (23.2% of the total variance), versus PC3 (22.8% of the total variance), which indicated phosphatase activity and metabolic index was significant correlated, and high correlation of ammonium and protease activity. Fur-

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thermore, the effects were more evident in *Raphanus* treatments than in *Avena*, suggesting the higher sensitivity of *Raphanus* to polymers treatment, which indicate biodegradation of polymers in *Raphanus* treatment has produced intermediate phytotoxic compounds.

Introduction

Mulching by putting a thin plastic film directly over the soil surface has become a standard technique for vegetable growers around the world (Vilaplana et al., 2010). In agricultural field and greenhouse cultivation, polymer materials are widely used for mulching, in order to protect humidity and stabilizing soil temperature, reduce germination time, weeds and plant diseases, provide early or out of season crop production for better market value produce, et cetera (Kijchavengkul et al., 2008). Despite all these advantages, there are several major concerns about costs of removal and disposal of the used plastics and environmental issues. Compare with the common (mostly polyethylene) plastic films, biodegradable films have been found to be a promising alternative for its potential of labor saving and environmentally friendly (Lamont, 1999). These biodegradable mulching films can be incorporated into the soil at the end of the crop season and undergo biodegradation by soil microorganisms (Wiles and Scott, 2006). However, the low-cost disposal of wild used biodegradable mulching films could be leaving them into soil (Kyrikou and Briassoulis, 2007). The residual of biodegradation plastic mulches can pose major concerns on its possible toxic effects on plant germination and growth as they were degrading in soil (Rutiaga et al., 2005). Only extensive analysis over the entire life cycle of a particular polymer application can assess eventual risks for the environment and plant health and, a selection of safer raw material can be made (Silvestre et al., 2011). The possible biodegradation of them are very important subjects with economic and environmental aspects (Snežana et al., 2017).

An aliphatic–aromatic copolyester of 1, 4-butanediol, adipic acid and terephthalic acid, called Ecoflex, has been recently reported as possible alternative to traditional plastics (Yamamoto et al., 2005). The Ecoflex degradability has been studied in compost material at high temperature showing a complete degradation in 22 days of incubation without adverse ecotoxicological effects (Witt et al., 2001). This lack of toxicity indicates that Ecoflex could be excellent for use in short-life products such as protective packing, film for foods and agricultural applications. Thus, at present work it was used as positive control sample together with filter paper (cellulose) for their environment friendly biodegradability.

The overarching goal of present work was to monitor the contamination soil interferent by the polymer materials, determine the metabolic changes in soil and effects on plant health. During incubation procedure of plants-soil-polymers mesocosm experiments, different biomaterials in powder was applied to agricultural soil in order to 1) evaluate the polymer films biodegradability and investigate the their monitoring management, make an assessment of polymer powder biodegradation effect; 2) seek for proper management to indicate the contamination soil, choose better bio-indicator to make soil infrastructure detection; 3) by means of the fates and effects of polymer pollutants examination, supporting practices of remediation in large-scale ecological system, therefore make the contribution to geochemical and biological processes, and the application of biodegradation materials in agriculture.

1. Experimental section

1.1. *Materials*

At present investigation, the extent biodegradation, potential eco-toxicological effects of synthetic biodegradable polymer materials were investigated under laboratory conditions. Two terrestrial plants, oat (*Avena sativa*, in this article use *Avena* or *A* as abbreviation) and red radish (*Raphanus sativum*, in this article us *Raphanus* or *R* as abbreviation), were used to evaluate the phyto-toxicity of polymers.

Different polymers in powder at the final concentration of 1% were added to the soil; the polymer treatments were: polyethylene (PE, Polimeri Europa Riblene FL30, white powder (density as 0.918–0.935 g/cm^3), CIBA specialty chemical oxo-biodegradable Linear low-density polyethylene (LLDPE), thermally degraded, supplied by BASF The Chemical Company (Germany)), polystyrene (PS, Polimeri Europa powder, white powder (density as 0.918–0.935 g/cm^3), EPI-INC. oxo-biodegradable PS, thermally degraded, supplied by BASF The Chemical Company (Germany)), Ecoflex (E, Ecoflex ® FBX 7011 is a biodegradable, statistical, aliphatic-aromatic copolyester consisting of 1,4–butanediol, adipic acid and terephthalic acid monomers, supplied by BASF The Chemical Company (Germany) as white powder), cellulose (CE) biomaterial was detected as reference treatment. Moreover, soil sample without any treatment was used as control(C). The monitoring of the mesoscale experiment consisted of soil sampling carried out after one days (T0), 35 days(T1), 45 days (T2) from the experiment set up.

1.2. *Methods*

The experiment at mesoscale level was carried out using pots with a diameter of 25 cm and a height of 10 cm (mesocosms) containing 1 kg of soil. The main characteristics of the soil in use are shown in Table 1.

The soil was treated with different polymers-PE, PS, Ecoflex and Cellulose -in powder at the final concentration of 1% of quality. Soil samples with Ecoflex and Cellulose were used as the positive control and which without any treatment were used as blank control.

Table 1 – Chemical, biochemical and microbiological parameters of the soil used in the experiment.

	Soil characteristics
Original soil umidity (%)	3.26±0.13
pH	6.93±0.27
Electrical conductivity (dS/m)	114±1.4
Total organic carbon (%)	2.50±0.03
Total Nitrogen (%)	0.323±0.01
NO_3^- (mg/kg)	234±6
NH_4^+ (mg/kg)	3.43±0.72
Available Cu (mg/kg)	77.8
Available Zn (mg/kg)	17.48
Available K (mg/kg)	323
Available Mg (mg/kg)	108
AvailableFe (mg/kg)	19.4
Available P (mg/kg)	27.5 ± 3.8
Total Cu (mg/kg)	64±7
Total Zn (mg/kg)	58±23
Total Ni (mg/kg)	63.6
Total Pb (mg/kg)	26.7
Total Cr (mg/kg)	41.9
Total Cd (mg/kg)	0.3
Total K (mg/kg)	6076±1067
Total Mg (mg/kg)	6890±583
Total Fe (mg/kg)	8395±1097
Total cultivable microbial population (UFC/g)	5.34E+07±7.31E+06
Pseudomonas (UFC/g)	4.1E+05±4.9E+02
β-glucosiadse activity (mg PNP/(kg•hr))	580
Dehydrogenase activity (mg INTF/(kg•hr))	2.75

The pots were incubated under controlled temperature (25 °C), light (100lux) and humidity: The moisture content of each soil–biomaterial mixture was adjusted as 50% Water holding capacity (WHC). The monitoring of the mesoscale experiment consisted of soil sampling carried out after 45 days from the experiment set up. Each soil sample was a composite of three sub-samples mixed, homogenized, sieved (2 mm) and stored dried at room temperature until chemical analysis and stored at 4 °C until biological analysis. Furthermore, two plant species *Avena* and *Raphanus*, were used to evaluate the phytotoxicity of the biomaterials. For this purpose, after 35 days from the experiment set up, 100 seeds of each plant species were sown uniformly in each pot and all mesocosms were maintained under controlled temperature, light and humidity (as mentioned before) for 10 days. The growth above ground was checked at the end of experiment (after 10 days of incubation); at this time the plants were harvested (whole plants) at the soil surface and the dry weight of shoot and root and the plant length were determined. The germination index was calculated by using equation as follow:

$$GI = \frac{\text{n.seed germinated test}}{\text{n.seed germinated control}} \times \frac{\text{length plant test}}{\text{length plant control}} \times 100 \quad (1)$$

GI: germination index, n: number of germinated seeds, test: test samples, control: control samples, length: the length of plants.

1.2.1. *Chemical parameters*

Electrical conductivity (EC) and pH were measured in 1:10 (W/V) aqueous solution. Total organic carbon (TOC) and total nitrogen (TN) content of soil were determined by RC-412 multiphase carbon and FP-528 protein/nitrogen determinator (Leco, USA), respectively. Ammonium and nitrate were determined with selective electrodes. Water soluble carbon (WSC) was determined spectrophotometrically at 590 nm by using the method of dichromate oxidation (Yeomans and Bremner, 1988; Garcia et al., 1991). Total and available elements were determined with Atomic Absorption Spectrometry (AAS). Available P content was determined by the Murphy and Riley method (Mur and Riley, 1962).

1.2.2. *Biochemical parameters*

The proteases are a group of hydrolytic enzymes connected to the nitrogen cycle; they catalyzed the hydrolysis of proteins in oligopeptides or dipeptides. The activity is determined through the concentration of NH_4^+which is released by deamination (Bonmatí et al., 2009). The ammonia released was determined through a NH_3 electrode.

Tests (0.5 g of soil with 2 mL of phosphate buffer 0.1 mol/L at pH 7 and 0.5 mL of substrate BAA (*N-α-Benzoil-L-α-Arginammide-Hydrochloride*) 0.03 mol/L) and controls (0.5 g of soil with 2 mL of phosphate buffer 0.1 mol/L at pH 7) were shacked in a thermostatic bath at 37 °C for 1 hr and half. Then the samples were brought to a final volume of 10 mL with bi-distilled water and centrifuged for 10 min at 1369.55 x g. The supernatant was read with a NH_3 electrode. The results are expressed in mg NH_3/(kg•hr).

The phosphatase catalyzes the hydrolysis of phosphoric ester to phosphate. The methodology consists in the determination of the release of para-nitrophenol (PNF) from the hydrolysis of the para nitrophenil-phosphate-esaidrate (PNP) (Tabatabai and Bremner, 1969; Speir and Ross, 1976). Tests (0.25 g of soil + 2 mL of maleate buffer 0.1 mol/L at pH 6.5 + 0.5 mL of PNP substrate 0.12 mol/L) and controls (0.25 g of soil + 2 mL of maleate buffer 0.1 mol/L at pH 6.5) were shaked in a thermostatic bath at 37 °C for 1 hr and half. 0.5 mL of substrate (PNP) was added also to the controls and they were made cold at 4 °C for 10 min, to block the reaction. Then the samples were brought to a final volume of 10 mL with bi-distilled water, and 0.5 mL of $CaCl_2$ 0.5 mol/L and 2 mL of NaOH 0.5 mol/L were added; samples were centrifuged for 10 min at 1369.55 xg. The supernatant was read with the spectrophotometer at a wave length of 398 nm. The optic densities picked up by the instrument are transformed in concentrations referred to a standard straight line obtained by the known concentration of PNF. The results are expressed in g PNF/(kg•hr).

Dehydrogenase (DH-ase) catalyze the oxidation of organic compounds. The reaction substrate consists in the organic substance, while the synthetic cofactor utilized for the measure of the dehydrogenise activity consists in the INT (*P-Iodio-Nitro-Tetrazolium-chloride*) which makes a chlorate product through reduction, INTF (*p-Iodo-Nitro-Tetrazolium-Formazano*) determinable with a spectrophotometric methodology. This enzymatic activity was determined using the methodology studied previously (García et al., 1993; Masciandaro et al., 2000). Tests (0.1 g of soil with 0.2 mL of INT substrate at 0.5% (in bi-distilled water) and 0.1 mL of bi-distilled water (to bring the

sample at the 60% of the field capacity)) and controls (0.1 g of soil with 0.3 mL of bi-distilled water (to bring the sample at the 60% of the field capacity)) were let rest for 20 h in dark place; the test-tubes were not covered because the INT prevails oxygen (the natural substrate of the dehydrogenase) as electrons acceptor. The INTF is the product of the oxidoreduction and it is insoluble in water; it was extracted through the addition of an extractant solution (tetrachloroethylene and acetone, 1:1.5). The solution was shacked for one minute and centrifuged at 1369.55 xg for 10 min. The supernatant was used for the spectrophotometric measure, using a wave length of 490 nm, referring it to the control. The optic densities picked up by the instrument are transformed in concentrations, express in mg INTF/(g•hr), referred to a standard straight line obtained by the known concentration of INTF.

1.3. *Statistics*

The STATISTICA 6.0 software (StatSoft Inc., Tulsa, Oklahoma, USA) was used for all statistical analysis. All numerical parameters before statistical analysis were normalized and auto scaled: the result for each variable is a zero mean and a unit standard deviation (Bilck et al., 2010).

Principal components analysis (PCA) is a multivariate statistical data analysis technique which reduces a set of raw data into a number of principal components which retain the most of the variance within the original data in order to identify possible patterns or clusters between treatments and variables (Carroll et al., 2004). All numerical parameters before statistical analysis were normalized and auto scaled: the result for each variable is a zero mean and a unit standard deviation (Latorre et al., 1999). The PCs were extracted by applying the principal of main axis method. Only component loadings >0.7 were considered for interpretation of the PCs. In PCA analysis, the data are decomposed into separate sets of scores and loadings for each of the two modes of interest (treatments and variables) and the whole variability of the data is explained in order to provide a clear and more interpretable visualization of data structure in a reduced dimension. In addition, PCA analysis gives information that can also be clearly presented graphically (Tsi et al., 2009).

2. Result and discussion

2.1. *Plant growth parameter analysis*

Seed germination and metabolic Index values are reported in Table 2 and Fig. 1. Compare with control result, the germination percentage of PS treatment were higher than PE, the values even higher than cellulose-spiked soil sample, however the different growth trend obtained, PE treatment result were higher than PS and Ecoflex, which indicated PE degradability with *Raphanus* was higher than *Avena*.

The germination of the *Avena* seeds seems not to be negatively affected by the application of biomaterials; From T0-T1, higher percentages of seed germination were generally observed in the treated with respect to the control soil. On the other hand, compared with controlled plants, seeds germination dropped from 72% to 62% with PE treatment and 55% under PS treatment. This result was coordinate with previous report that compare with PS, PE degradation in soil have positive effect on seed germination (Miraftab et al., 2003; Tan et al., 2008).

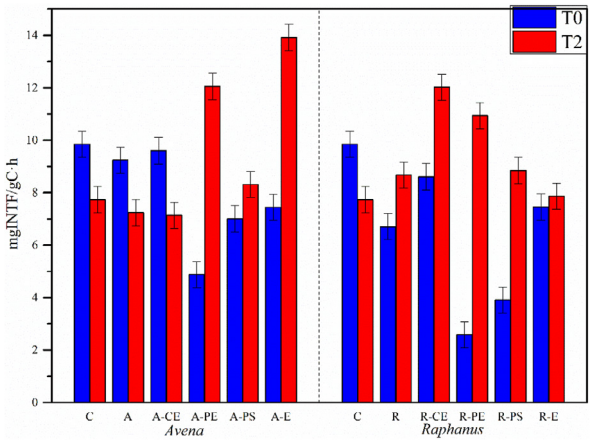

Fig. 1 – Metabolic Index of soil samples. Values in brackets are standard errors of the estimate ($p < 0.05$). The significant difference analysis results of *Avena* data: $F = 0.22$, *P*-value=0.94, *F*-crit=5.05. The significant difference analysis results of *Raphanus* data: $F = 0.62$, *P*-value=0.69, *F*-crit=5.05.

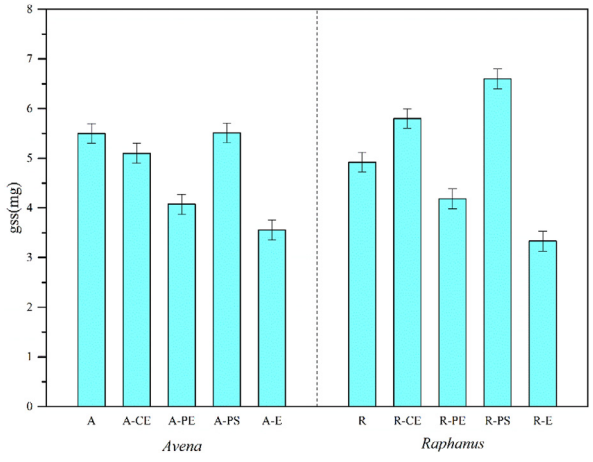

Fig. 2 – Plant biomass of mesocosm. Values in brackets are standard errors of the estimate ($p < 0.05$). The significant difference analysis results of data: $F = 9.67$, *P*-value=0.02, *F*-crit=6.39.

The plant growth of *Avena* and *Raphanus* were reported in Table 3. The plant length was measured every 3 days during incubation. Among two plant species, plant height showed no difference at T2. The lower value of the control *Avena* growth in both experiments (PE and PS treatments) confirms its stimulation by biopolymers application, while the higher growth of the control *Raphanus* confirms its inhibition by biopolymers.

Moreover, same differences about the seed germination trend in the different treatments were appeared. Plant biomass and seed germination rate were elucidated by the end of two experiments (Figs. 2 and 3).

Table 2 – Biochemical and microbiological parameters of the soil with treatment with treatment at beginning (T0) and the end(T2).

	Percentage of seeds germination		Average Plant Length (mm)		Plant biomass (g)		Protease activity (mg NH_3/(kg•hr))		Phosphatase (mg PNP/(kg•hr))		DH-ase (mg INTF /(kg•hr))	
	T0	T2	T0	T2	T0	T2	T0	T2	T0	T2	T0	T2
Control	–	–	–	–	–	–	17.50	16.16	254.95	226.12	3.59	3.52
Avena	30	67	9.90	106	0	5.50	20.14	20.21	211.89	233.61	3.61	2.83
Cellulose+*Avena*	46	73	10.1	103	0	5.10	23.05	24.26	227.08	240.36	4.27	4.63
PE+*Avena*	40	74	10.2	104	0	4.08	18.99	20.75	206.89	257.35	3.27	5.48
PS+ *Avena*	74	85	10.0	132	0	5.51	24.67	23.59	213.49	280.59	3.27	3.79
Ecoflex + *Avena*	52	77	10.1	115	0	3.56	16.36	14.94	220.08	190.64	4.27	4.54
Raphanus	72	92	10.0	53.9	0	4.92	18.25	16.29	201.89	230.87	3.42	3.20
Cellulose+*Raphanus*	69	82	10.0	39.3	0	5.80	22.10	20.07	243.47	452.01	4.57	4.70
PE+*Raphanus*	62	70	9.8	57.1	0	4.19	22.44	20.07	239.47	313.07	2.01	4.63
PS+*Raphanus*	55	80	10.0	49.0	0	6.60	15.75	16.83	181.10	276.09	2.75	3.46
Ecoflex+*Raphanus*	59	65	10.0	45.4	0	3.33	18.72	18.39	287.84	181.30	3.23	3.41

Values in brackets are standard errors of the estimate ($p < 0.05$).

Table 3 – Chemical parameters of the soil with treatment at beginning (T0) and the end (T2).

	pH		Electral Conductivity (μS/cm)		Water soluble carbon (WSC) (mg C/kg)		NO_3^-/NH_4^+		Nitrate(mg NO_3^-/kg)		Ammonia (mg NH_3/kg)	
	T0	T2	T0	T2	T0	T2	T0	T2	T0	T2	T0	T2
Control	7.1	7.2	106.3	133	466	455	18.26	145.91	225.12	1375.09	12.33	1.20
Avena	7.04	7.31	109.4	95.7	391	391	11.18	12.62	178.64	140.26	15.98	11.12
Cellulose+ *Avena*	7.02	7.22	157.3	118.9	445	649	14.22	10.79	229.97	158.44	16.18	14.69
PE+ *Avena*	7.17	7.21	123	118.3	670	455	34.75	28.21	263.23	296.49	7.58	10.51
PS+ *Avena*	7.07	7.18	128.1	125.7	466	455	15.3	22.89	320.23	314.69	20.93	13.75
Ecoflex + *Avena*	7.02	7.1	116.9	51.1	573	327	14.53	61.56	170.33	92.55	11.72	1.50
Raphanus	6.98	7.43	112.1	125.8	531	370	12.26	42.59	188.45	421.81	15.37	9.90
Cellulose+*Raphanus*	7.11	7.25	108.4	142.9	531	391	6.24	154.04	126.92	1229.31	20.33	7.98
PE+*Raphanus*	7.08	7.27	82.4	136.5	777	423	24.17	9.48	183.07	144.68	7.58	15.27
PS+*Raphanus*	6.95	7.28	114.5	117	702	391	9.14	86.50	177.50	620.24	19.42	7.17
Ecoflex +*Raphanus*	7.14	7.19	99.4	120.1	434	434	15.23	90.88	175.44	679.31	11.52	7.47

Values in brackets are standard errors of the estimate ($p < 0.05$). The significant difference analysis for pH results of *Avena* data: $F = 11.2$, P-value=0.03, F-crit=7.71. The significant difference analysis results for pH of *Raphanus* data: $F = 10.71$, P-value=0.03, F-crit=7.71. The significant difference analysis for E.C results of *Avena* data: $F = 4.32$, P-value=0.11, F-crit=7.71. The significant difference analysis results for E.C of *Raphanus* data: $F = 7.93$, P-value=0.05, F-crit=7.71.

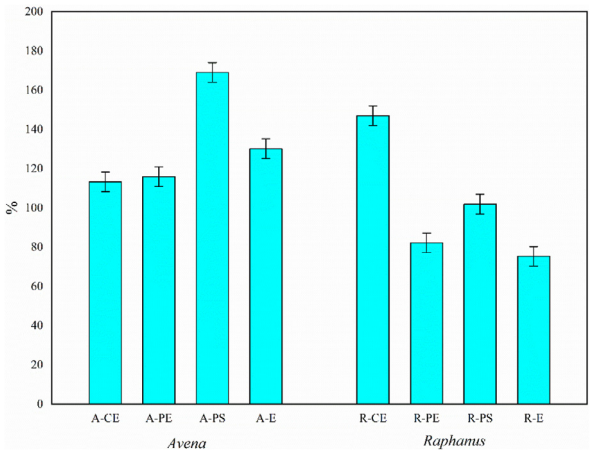

Fig. 3 – Plant germination index. Values in brackets are standard errors of the estimate ($p < 0.05$). The significant difference analysis results of data: $F = 0.69$, P-value= 0.62, F-crit=9.28.

In case of *Raphanus*, same grown trend with polymer treated soils were decreased in weight compare with control. The significant difference analysis of biomass results was: $F = 9.67$, P-value=0.02, F-crit=6.39. These data indicated two plant species growth behaved different with different polymer treatments, polymer materials powder has shown little inhibit effect on soil plant biomass even this effect was weak. However, among all treatments, the PE biomass behaved approximate value as control sample in both plants, this result imply negative effect on soil plant biomass of PE powder was less than other polymer materials. Thus, it got conclusion that the application of biomaterials should carry out pretreatment process before their cycling biodegradation in open environment (Wu et al., 2018; Valentin and Irina, 2018). The process of second experiment (from T1-T2) showed almost similar trends of plants weight which compared with first experiment's previous biomass values on day 10. The stable humidity condition has dramatically affected the plant growth (Anna and Irene, 2018), the PS still kept the same weakly negative effect on plant weight. Conversely, after 10 days, the PS and PE promoted soil microorganism's activity and soil enzymes activity, thus the plant biomass were increased in both plants. In conclusion this phenomenon indicated that *Raphanus* was more sensitive than *Avena* in soil constitution and environment. Therefore, in order to give assessment of eco-toxicological effect during short period, *Raphanus* should be considered as a better indicator.

2.2. *The pH value and the electrical conductivity (μS/cm) value of soil samples mixed with different polymers at different time*

The pH values and Electoral Conductivity (E.C) were showed in Table 3. The significant difference analysis for pH results of *Avena* was $F = 11.20$, P-value=0.03, F-crit=7.71, The pH results of *Raphanus* were $F = 10.71$, P-value=0.03, F-crit=7.71. These results indicated in both plant soil, pH value was significant influenced by different polymer treatments. pH values of polymers treated soil sample's all behaved significant increase from T1 to T2, while the significance for *Avena* performed higher than *Raphanus* (with same F-crit value, F-value of *Avena* was 11.2, higher than 10.71 as *Raphanus*), in both case PE reached lowest point at T2, while the control sample of *Avena* and *Raphanus* all behaved slightly alkaline. The significant difference analysis for E. C results of *Avena* was $F = 4.32$, P-value=0.11, F-crit=7.71, and results for E. C of *Raphanus* was $F = 7.93$, P-value=0.05, F-crit= 7.71. These results showed for two plants, the E.C value of *Raphanus* performed apparent increasing at T2, but interesting phenomenon was E.C value behaved inhibited in *Avena* soil from T0 to T2. Moreover, in both case E.C values of Ecoflex treatment has more significant effect in mesocosm.

The pH values change in biopolymers soil solutions indicated that the high biodegradability of polymers in plant soil matrix, the H^+ active acidity promotion probably reveal that LDPE Polypropylene-$[CH_2–CH(CH_3)]_n$-followed by chain scission to yield aldehydes and carboxylic acids by biochemical reaction, which coursed by soil microorganisms and enzyme during degradation. On the other hand, from results analysis, pH and E.C value of PS was changed but did not very significant as PE did, it indicated that polymers still do not lead to complete break down of whole olefins key in the benzene ring structure, except part of alkane bond scission, which stability probably because their petroleum-chemical industry manufacturing process (Kaplan et al., 1979), therefore its solid powder biodegradability performed corresponding complexity. Anyway, *Raphanus* behaved sensitive to E.C value, especially for the PE treated soil, as a high sensible indicator.

2.3. *Water soluble carbon, ammonia level and heavy metals of soil sample in the polymer sample*

The WSC results were shown in Table 2. This parameter is substances as indicators of the decomposition process and the microbial in the soil (Charest et al., 2004). Addition of polymer powder absolutely change the nutrition distribution of initial balance of soil system during polymers biodegradation by microorganisms. The PE and PS have dramatically increasing WSC of *Avena* plant soil at the end of experiment, higher than control sample and cellulose, also other polymers behaved promote trend curves,which means with polymers degradation, more and more alkane or alkylene backbone were breakdown to the initial monomer, therefore carbon can release from polymers and utilized by the soil microorganisms and enzymes as carbon row source, the microorganisms feed on them in their metabolism while the enzyme have part function of catalyst driver, so the whole biodegradation system was increased correspondingly. We noticed that the degraded polymers seem have more significant positive effect on the soil samples WSC, which phenomenon imply the polymers after degradation by photo explosion and oxidation, the carbon will get more possibility breakdown in soil during compost procedure. This interesting result may make positive sense to the agriculture and the polymer dispose technique.

Table 2 shows the ammonia level of different polymer soil sample at three in dependent sampling times respectively. Similarly, as results have been mentioned above of WSC value, the Ammonia level was promoted in various degrees,

especially in *Avena* growth soil with PE treatment. In case of *Raphanus*, it appeared obviously increasing for the average value of polymers. NH_3 content in soil were decreased at T2, it means company with both plants growth were promoted during experiment, nitrogen in the soil exists from an organic state (most of which are nitrogen compounds that cannot be directly absorbed and utilized), after being decomposed by microorganisms, it is converted to inorganic nitrogen and then used by crops. From the result, the ammonia has increase trend with PE treatment while decrease trend with PS treatment, which probably means PE powder biodegradability was fast than PS, thus it has negative effect on inorganic nitrogen transformation. And also, the Ecoflex sample did not changed a lot compare with the control sample, means it might be complete degraded during experiment procedure. The variability of Ammonia was a synthetic reaction, which should consider the nutrition distribution in the soil system, the nitration and denitrification reaction between soil microorganisms and soil biomass, and the respiration of the plant root and the nutrition recycling by the root system metabolism. The denitrification reaction is a kind of reduction of the Nitrate, Nitrate absorption and utilization of micro-organisms (denitrifying bacteria-most of them are heterotrophic bacteria) and plants, there are two completely different purposes, one is using one of the nitrogen as nitrogen source, known as the assimilation of nitrate reduction: $NO_3^- \rightarrow NH_4^+ \rightarrow$organic nitrogen (Vance-Harris and Ingall., 2005; Indu et al., 2019; Shimura et al., 2000).

The denitrification reaction can be described as follow equations:

$$C_6H_{12}O_6+12NO_3^- \rightarrow 6H_2O+6CO_2+12NO_2^-+Q \quad (2)$$

$$CH_3COOH+8NO_3^- \rightarrow 6H_2O+10CO_2+4N_2+8OH^-+Q \quad (3)$$

$$5S+6KNO_3+2H_2O \rightarrow 3N_2+K_2SO_4+4KHSO_4 \quad (4)$$

According to the equation above, it is can make a hypothesis that carbon source decides the NH_3 level in the soil sample. In this denitrification reaction, without any addition of nutrition and without any change of experiment conditions, the only impetus to push forward whole reaction depend on the increase of carbon nutrition of soil- glucose ($C_6H_{12}O_6$), meanwhile the $C_6H_{12}O_6$ increasing was depend on the addition carbon source, and the only source of carbon at present experiment just can come from biodegradation of polymer powder caused by the biochemical reaction in soil, correspondingly, the inferred conclusion of hypothesis is the polymer biodegradation lead to the NH_3 level promotion of polymer soil.

The heavy metal analysis was measured in this experiment, and the sampling results were showed in Table 1 and Fig. 4. From the values Cd was higher than other heavy metals. And in original soil, the heavy metal average level was higher than the polymers.

The heavy metal in the soil and plant have very important impact on microorganisms diversity and activity (Li et al., 2017; Sharma et al., 2007; Adília and Pampulha, 2006; Mishra and Tripathi, 2008), and microorganisms have straight effect on the soil enzyme and biodegradation of polymers. The result of significant analysis showed that except the Cr and some individual sample as *Raphanus* control, all other heavy

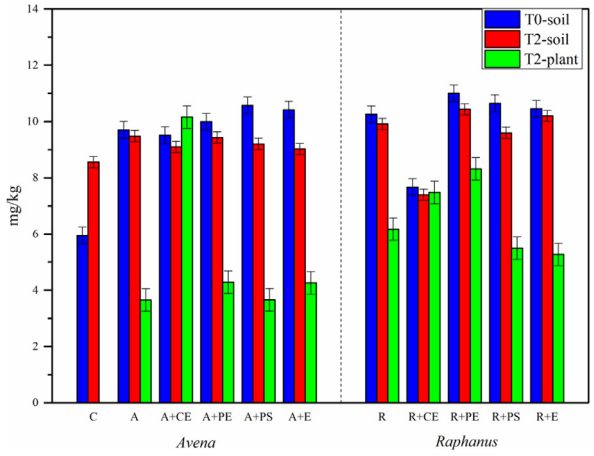

Fig. 4 – Heavy metals of soil samples and plants. Values in brackets are standard errors of the estimate ($p < 0.05$). The significant difference analysis results of *Avena* data: $F = 0.61$, *P*-value=0.68, *F*-crit=6.39. The significant difference analysis results of *Raphanus* data: $F = 57.88$, *P*-value=0.0009, *F*-crit=6.39.

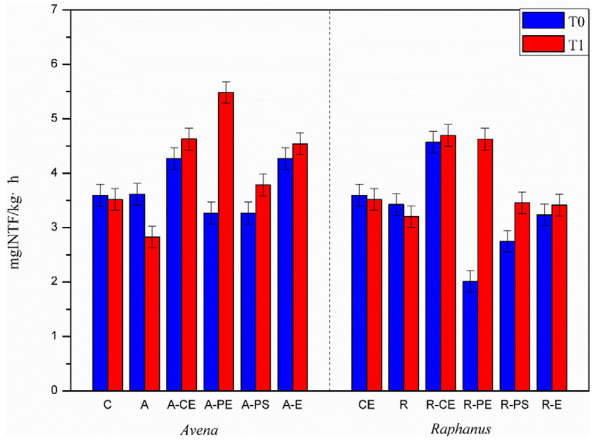

Fig. 5 – Dehydrogenase activity of soil samples. Values in brackets are standard errors of the estimate ($p < 0.05$). The significant difference analysis results of *Avena* data: $F = 1.21$, *P*-value=0.42, *F*-crit=5.05. The significant difference analysis results of *Raphanus* data: $F = 1.10$, *P*-value=0.46, *F*-crit=5.05.

metals were dramatically decreased at T2 (Fig. 5), which indicated that the plant cultivation activity have the positive effect on the heavy metal decrease of the soil, which help the heavy metal transform of polymers during their biodegradation, thus further support the dynamic of degradation. Except for the Cd, all other heavy metal level in the polymers soil were increased compare with original, this phenomenon has positive meaning of the polymer depose process with plant growth as well as the polymers addition increased the heavy metal level. The high level of Cr showed this kind of heavy metal was a long-term residue in the soil and difficult to remove from soil. Meanwhile, the heavy metal level remarkably higher than

other samples, this imply the sensibility of *Raphanus* again in this experiment.

At the end of the experiment, the results showed that compared with the control samples, heavy metals levels increased in both plants, and compare with the increase content in the plants, heavy metals lever in the soil behaved a downward trend, this upward trend in plants is basically the same as the downward trend in soil. This result shows that plants have a certain absorption and repair effect on heavy metals in the polymer-soil mixed culture medium containing heavy metals. The phytoremediation technology of heavy metal contaminated soil should be suggested used in the research of heavy metal contaminated areas.

2.4. *Nitrate level, protease, phosphatase and dehydrogenase activity in soil*

The nitrate level of polymer treated soil was showed in Table 3. The NO_3^-level in polymer soil sample was increased at different sampling time respectively. Control sample average value was highest among all samples. The polymer-PE, PS were increased dramatically at T2, also PS resistant high level at T2. Cellulose sample and Ecoflex all performed increasing growth at T2 in distinct degrees, especially for the *Raphanus* compare with T0. The lids were used to cover pots in this experiment for purpose of keep the resistant WHC of soil, at same time, air condition resistant kept the N_2 level inside the pot was limited, thus the N cycle rate was decrease since T1, and lead to the N level was decrease compare to the T0. The NO_3^-level of *Avena* samples were decreased while values were increased dramatically in *Raphanus* during procedure, which because of different nitrogen need by different plants species. The majority of mineral N in the soil is in the form of "NO_3^--N", which exist in soil solution and water transported easily in most condition. A rapidly growing and transpiring crop, such as corn, may acquire approximately 80% of its N through mass flow (Wim and Bar-Yosef et al., 2019; O'Reill, 2009).

Protease (also termed peptidase or proteinase) breaks down proteins. Protease is an enzyme that conducts proteolysis, that is, begin sported in catabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein. The result was showed in Table 2. The protease activity of sample all increased at T2, expect the cellulose treated soil and control soil in *Raphanus* case, which indicated this protein increased with the polymer's biodegradation by the reaction under the soil microbes' metabolism. The protein level was increased by the enzyme activity in soil. The interesting situation showed the polymers have good possibility of biodegradation in soil at present experiment. The cellulose plus *Raphanus* and control sample protease activity were decreased means the enzyme in both cases was inhibited without added any carbon source to promote the soil biomass.

The phosphatase activity of polymer soil sample at different times was showed in Table 2. All testing samples kept decreasing during biodegradation from T0 to T2. PE and PS all performed high activity of phosphatase at T1(sowing time). Phosphatase activity play an important role in soil enzyme activity and total microbial population (fungi, bacteria and actinomycetes), because it resistance of dilution function to the soil nutrition distribution (Vinotha et al., 2000). The microorganism will determine the soil biomass and the activity during polymer biodegradation. Thus, the phosphatase activity is an important parameter to imply the polymer soil biodegradation. The gradually decreasing of the phosphatase activity showed the dilution function of this enzyme was inhibited by the increase the carbon course, so it indicated that the polymer was break down during the experiment.

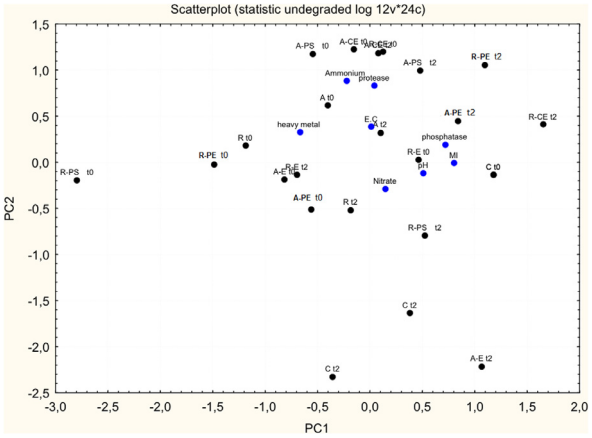

Fig. 6 – Statistics analyzes about Biplot PC1 versus PC2.

Dehydrogenase activity of soil samples are showed in Fig. 5 and Table 2.

It is an enzyme that oxidizes a substrate by transferring one or more hydrides (H^-) to an acceptor, usually NAD^+/$NADP^+$ or a flavin coenzyme such as FAD or FMN. During biodegradation, Dehydrogenase decreased with time in blank soil and control soil with both plants' growth. However, all other polymer treated soil sample appeared promotion on dehydrogenase activity in both plants, especially for the PE treatment. Which indicated the vast majority redox reactions of organisms are under dehydrogenase and oxidase catalytic manner, after dehydrogenase catalytic oxidation, substance finally be oxidized by oxygen through the electron transport chain, meanwhile, adenosine triphosphate (ATP) was generated by oxidative phosphorylation, which is the main way to obtain energy to object heterotrophic organisms, thus this interesting result imply that soil microorganisms (heterotrophic organisms) activity and population were increasing by the biochemical reaction during the biodegradation of the polymers in soil. Correspondingly, the increasing of dehydrogenase activity indicated the polymer was degradation rapidly in the soil.

2.5. *Effects of polymers on soil properties and plant growth*

The Figs. 6 and 7 are the statistics analysis of all parameters Principal Component Analysis (PCA).

The chemical and biological results were studied using the Principal Component Analysis (PCA). PCA is a multivariate statistical data analysis technique which reduces a set of raw data into a number of principal components which retain the most of the variance within the original data in order to iden-

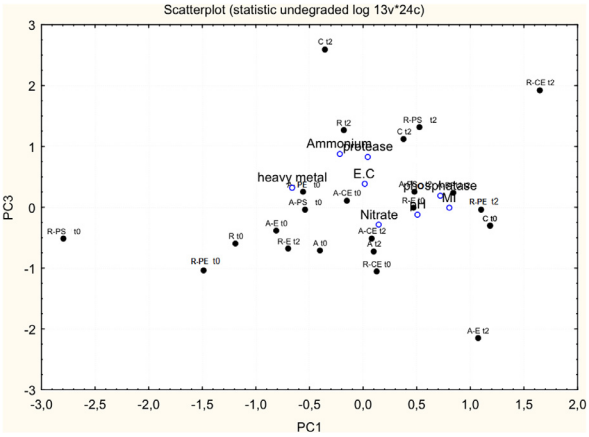

Fig. 7 – Statistics analyzes about Biplot PC1 versus PC3.

Table 4 – Principal component analysis (PCA) of paremeters.

	PC1	PC2	PC3
Ammonium	−0.220	0.881	−0.089
Nitrate	0.148	−0.291	0.866
protease	0.044	0.832	0.088
phosphatase	0.719	0.193	0.453
heavy metal	−0.665	0.327	−0.105
pH	0.509	−0.117	0.410
E.C	0.012	0.384	0.813
MI	0.802	−0.008	−0.118
Variance explained (%)	24.2	23.2	22.8
Total variance explained (%)	70.2		

tify possible patterns or clusters between treatments and variables. The PCs were extracted by applying the principal of main axis method. Only component loadings >0.7 were considered for interpretation of the PCs. In PCA analysis, the data are decomposed into separate sets of scores and loadings for each of the two modes of interest (treatments and variables) and the whole variability of the data is explained in order to provide a clear and more interpretable visualization of data structure in a reduced dimension. In addition, PCA analysis gives information that can also be clearly presented graphically (Carroll et al., 2004).

Tables 4 and 5 are the parameters correlation analysis of all test parameters of two plants, *Avena* and *Raphanus*.

From the tables, PCA analysis isolated three principal components (PC) (Total variance explained: 70.2%) covering variables related to chemical and biochemical parameters of the different treatments (Table 3). The highest loadings of the first PC (PC1, 24.2% of the total variance) included the phosphatase activity and the metabolic index (DH-ase/WSC) which show positive correlation (Fig. 1), meaning that the carbon cycling was activated and was able to sustain microbial activity. The second PC (PC2, 23.2% of the total variance) included the ammonium and the protease activity, indicate the activation of nitrogen cycling. Finally, the third PC (PC3, 22.8% of the total variance) included the nitrate and the electrical conductivity demonstrating also that nitrate contributed to soil electrical conductivity.

PC1 and PC2 were chosen for the interpretation of the biplot of scores and loadings (Figs. 6 and 7), in order to draw conclusions about the effects of polymer materials application on the soil chemical and biochemical characteristics.

The treatments, at T1 sampling time were shifted on the right of the biplot with respect to T0 and resulted located nearer to the biochemical parameters. This indicated a stimulation over time of the phosphatase activity (Table 2) and metabolic index (Fig. 1), which were significant on PC1 axis.

On the contrary, the treatments at T2 were generally shifted towards negative values of the second principal component (PC2), which represents the variation of ammonium and protease activity, thus indicating a decrease of these parameters.

Considering the different treatments, PS and PE at T0 are located on the left negative side in the biplot (PC1 axis) indicating a negative effect of these polymers on microbial metabolism. After forty-five days (T1) incubation, such negative effects resulted less evident, showing the treatments a cluster in the positive side of the biplot. The correlation coefficients among the parameters have been calculated separately for *Raphanus* and *Avena*, to better relate soil metabolic processes and plant germination and growth (Fig. 3).

Plant germination and growth. *Raphanus* plant biomass correlated negatively with the heavy metals and positively with the nitrate. Ammonium seems to cause some toxic effect on germination (even low significant) and plant growth being the correlation negative. Interesting are the highly significant coefficients among metabolic index MI with protease and phosphatase, which demonstrate that soils did not alter their microbiological conditions and P-N nutrients cycling, being these hydrolytic enzymes involved in the N-P metabolism. *Avena* seems to modify soil biological characteristics more than *Raphanus* did, probably due to its extended roots. In fact, negative correlation was found between plant biomass growth and the metabolic index MI, while positive significant correlation was found with parameters governing N-cycling, i.e. protease and ammonium and, to a lesser extent, with phosphatase. The fact that protease, easily available carbon (WSC) and ammonium were correlated, suggested that carbon metabolism was related to and sustained by the nitrogen metabolism; this could make easier the polymers biodegradation even in an environment apparently altered in microbial activity. It is known that extracellular protease and phosphatase are often involved in sustaining metabolic processes occurring in extreme soil environments where microbial life is scanty.

The all parameter analysis was determined by statistic method which was showed in Figs. 6, 7 and Table 5. From the PCA analysis we can get the conclusion that Polypropylene and polystyrene in the degraded form, being very near in the biplot, seem to affect soil properties regardless of plat specie, instead Ecoflex, and to a less extent cellulose biomaterial, affect soil properties in relation to plant species. Ecoflex is isolated in the biplot from all other cases, suggesting more meaningful impact on soil chemical and biochemical properties and on plant germination and growth.

Table 5 – Correlation analysis about testing paremeters of *Avena* and *Raphanus*.

		Avena											
		WSC	Amm	Nitrate	protease	phosph	Heavy-Me	DH-ase	pH	E.C	M. Index	plant	GI%
Raphanus	WSC	–	0.599	0.037	0.715	0.419	0.293	0.25	0.265	0.629	−0.569	0.405	−0.165
	Amm	−0.254	–	−0.535	0.977	0.755	0.761	0.076	0.504	0.406	−0.46	0.817	0.079
	Nitrate	−0.639	−0.314	–	−0.396	−0.028	−0.626	−0.268	−0.012	0.542	−0.299	0.259	0.519
	protease	0.017	0.626	0.087	–	0.802	0.634	0.105	0.428	0.536	−0.522	0.758	0.172
	phosph	−0.226	−0.099	0.689	0.58	–	0.288	0.043	0.355	0.75	−0.425	0.623	0.45
	Heavy-Me	−0.36	0.653	−0.365	0.024	−0,510	–	−0.214	0.774	0.023	−0.436	0.711	−0.533
	DH-ase	0.023	0.358	0.231	0.95	0.822	−0.275	–	−0.483	−0.067	0.631	−0.697	−0.131
	pH	−0.801	0.155	0.271	−0.282	0.048	0.394	−0.143	–	0.423	−0.707	0.697	−0.468
	E.C	−0.127	−0.154	0.364	0.313	0.817	−0.395	0.681	0.326	–	−0.667	0.636	0.178
	M.Index	−0.363	0.399	0.489	0.823	0.872	−0.135	0.922	0.163	0.691	–	−0.955	0.023
	Plant	−0.696	−0.777	0.851	−0.258	0.518	−0.714	0.054	0.384	0.393	0.292	–	0.206
	GI%	−0.579	−0.65	0.931	0.236	0.857	−0.511	0.444	0.196	0.657	0.673	0.67	–

3. Conclusion

By applying indicator plants *Avena* and *Raphanus*, two mesocosms behave significant different from experiment set up. Among them, The F-value of significant analysis of biomass, pH, heavy metal and electoral conductivity of *Raphanus* were 9.67, 11.2, 10.71, 57.88 and 7.93 after 10 days incubation, all more than "F-crit" values respectively, and theirs *p* values all less than 0.05, which indicated these parameters all behaved significant different from experiment set up (T0) to the end, among them, biomass dramatically increase in both soil showed the polymer biodegradation property in soil, pH and E.C value of PS was changed but did not behaved very significant as PE did, it means biodegradation was fast in PE than PS, besides, heavy metals were dramatically decrease in soil at the end of experiment implied the plant absorption of metal may help decrease heavy metal toxicity of soil.

The stable humidity condition has dramatically affected the plant growth, PS and PE kept the same weakly negative effect on plant weight. Conversely, after incubation, the PS and PE behaved their promoted effect on soil microorganisms and soil enzymes, thus the plant biomass obtained were increased in weight both of *Avena* and *Raphanus*. Moreover, the increase value at T2 of Dehydrogenase activity (0.84 higher than average value for *Avena* & 0.91 higher for *Raphanus*), Metabolic Index (3.12 higher than average value for *Avena* & 3.81 higher for *Raphanus*) means during experiments, soil enzyme activity was promoted by polymer biodegradation for its heterotrophic organisms' energy transportation was stimulated. From PC results indicated phosphatase activity and metabolic index was significant correlated, and good correlation between ammonium and protease activity. Furthermore, the effects were more evident in *Raphanus* treatments than in *Avena*, suggesting the higher sensitivity of *Raphanus* to the polymer's treatment, which indicate biodegradation of polymers in *Raphanus* treatment has produced intermediate phytotoxic compounds. Anyway, this phenomenon indicated that the *Raphanus* was sensitive than *Avena* in soil constitution and environment. Therefore, in order to give assessment of eco-toxicological effect during short period, *Raphanus* should be considered as a better indicator.

Polymers stimulated soil metabolic potential with time, thus enhancing better polymer biodegradation; microbial stimulation was due to nitrate releasing following polymer degradation. These effects were more evident in *Raphanus* treatments than in *Avena*. *Raphanus* should be considered as a best indicator for its sensitivity. The soil enzyme phosphatase and dehydrogenase activities increased with the polymer's biodegradation, and this was more evident in *Raphanus*. Therefore, it is supposed that *Avena* is less sensitive than *Raphanus* to soil metabolism.

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