Annals of Agricultural & Crop Sciences

Open Access

Austin Publishing Group

Research Article

Stress Effect Induced by Microplastics Coupled with Acid Rain, on Garden Cress, During Short and Long Time: Two Exposures in Comparison

Pignattelli S[1], Broccoli A[2]* and Renzi M[3]

[1]Laboratory of Environmental and Life Sciences, University of Nova Gorica, Vipavska cesta 13, SI -5000, Rožna Dolina (Nova Gorica), Slovenia

[2]Bioscience Research Center, Via Aurelia Vecchia, 32, 58015, Orbetello (Grosseto), Italy

[3]Department of Life Sciences, University of Trieste, Via E. Weiss, 2, 34127, Trieste, Italy

***Corresponding author:** Andrea Broccoli, Bioscience Research Center, Via Aurelia Vecchia, 32, 58015, Orbetello (Grosseto), Italy

Received: August 06, 2021; **Accepted:** September 02, 2021; **Published:** September 09, 2021

Abstract

Aim: Plastics, and acid rain, are the results of the anthropogenic activities; although, the injuries caused by acidic precipitation to plants are well known, what happen if acid rain is coupled with microplastics pollution?

Methods: In this experiment, we simulated, under controlled condition, a contaminated area from four different kinds of Microplastics (MPs): Polyethylene (PE), Polyvinylchloride (PVC), commercial mixture (PE + PVC) and Polypropylene (PP) subjected to acidic precipitation on *Lepidium sativum*, both in short then long exposures. At the end of experimentation were analysed: i) plant biometrical parameters (percentage inhibition of seed germination, plant height, leaf number and fresh biomass productions); and ii) oxidative stress (e.g., levels of hydrogen peroxide, glutathione, and ascorbic acid). On plant subjected to long exposure are quantified also: lipidic peroxidation through Malondialdehyde (MDA) concentration, Aminolevulinic Acid (AlA), pigments (Chl-a, Chl-b, carotenoids) and soluble sugars.

Results: Our results suggest that root system is more sensitive to MPs coupled with acid rain then above ground biomass. Furthermore, in short exposure toxicity begin with PE+ treatments to shift towards PVC+ toxicity in long exposure at radical level.

Conclusion: Our experiment demonstrates that different kinds of microplastics supplied with acid rain are able to interfere in different ways both in short and in long exposure, but also at shoot and root levels, on garden cress.

Keywords: Polypropylene; Polyethylene; Polyvinylchloride; Microplastic; Acid Rain; Oxidative burst, *Lepidium sativum*

Introduction

Microplastics, are emerging as new generation pollutants, they are ubiquitous in different environments, such as atmosphere, water, and soil [1]. At chemical level, microplastics are synthetic polymer-based materials used daily in human activities [2], their global production was estimated to be 348 million tons in 2018 [3]. Plastic litter are classified to its size, and the term "microplastics" refers to all plastics with size less than 5mm (<5mm). They are divided in primary and secondary; the first ones are directly manufactured in a micro-size range, the seconds are generated by micro fragmentation of a larger plastic waste [4]. Often, is a common practice use the sewage sludge for fertilize purposes, in fact it is estimated that in the Europe farmlands are released around 63,000 tons of microplastics annually [5]. For this reason, the agricultural lands are considered the major microplastics pollution source, because they can contain primary microplastics by the application of biosolid resulting from the sewage sludge, and secondary microplastics obtained by the fragmentation of plastic mulching film or others plastic materials used for agricultural aims [6]. Due to low light and oxygen conditions, when arrive in soil plastics can resist more than 100 years, and so interact at fauna and structural level, impairing bulk density, water holding capacity, and nutrition contents. Such changes can reflect negatively in a plant growth and yield [2,5]. Regarding as microplastics fate and transport within the soil, it can occur by soil tillage, earthworms, and springtails both in horizontal than vertical level [7].

Normally, when toxicants enter in agroecosystem, soil living organisms are the first to be affected, plants included in this frame, the first organs that can be in touch with pollution are roots [8]. Roots play an important role not only for the plant growth and development, but also anchoring them to the soil, in the uptake of water and nutrient from soil solution [9]. When they sense toxicants, the first response consists in the production of defense compound; but if the stress condition persists, they will result in a stunted growth and development; that which in turn will be reflected also, in above ground organs [8,9]. Abiotic stress effect at roots level, are well known and studied, such as drought, heavy metal or salinity [8], but on the other hand still is little known on microplastics on plants and in particular at root level.

Another new generation contaminant due to anthropogenic activities is acid rain; it is resulted from the fuels combustion that release in atmosphere sulphur dioxide (SO_2) and nitrogen oxides (NOx). These oxides react with water and consequently sulphuric and nitric acid form this acidic precipitation [10]. To be considered

Citation: Pignattelli S, Broccoli A and Renzi M. Stress Effect Induced by Microplastics Coupled with Acid Rain, on Garden Cress, During Short and Long Time: Two Exposures in Comparison. Ann Agric Crop Sci. 2021; 6(6): 1094.

acid, precipitations must have a pH value lower than 5.6 [11]. Due to its harmful for ecosystem, acid rain is considered together with global warming and ozone depletion as the biggest environmental disaster for the functioning of ecological systems [12]. Such as each environmental stressor, also acid rain induces injuries in plants, for instance it can impair the photosynthetic machinery that, which in turn, causes a reduced growth and biomass production and an over production of reactive oxygen species (ROS; [13]. However, at ground level, acid rain can cause a general soil acidification, and the direct consequence is that the essential mineral elements are not, anymore, give available for plants uptake and translocation, and consequently for their growth [12].

Due to their sessile life style, plants are not able to escape from environmental stress; for this reason, they have developed, during their evolution course, some defense mechanisms of response at metabolically level, to counteract these stress factors [14]. The first line to defense consists to trigger signal molecules production, such as Reactive Oxygen Species (ROS) that have the aim, in turns, to activate antioxidants production. However, if the ratio ROS/antioxidant is unbalanced in favor of ROS production, an impairment of plant growth, photosynthesis, and biochemical processes can also occur [15]. To evaluate and understand the combined effect resulted from microplastics and acid rain, we used *Lepidium sativum L.*, also known as garden cress. It is a fast-growing annual herbaceous plant belonging to the *Brassicaceae* family with a global diffusion; it is widely used both as an edible plant than phototherapeutics purposes [16]. Its high sensitivity to phytotoxic substances makes it suitable for biological test [17,18].

The aim of this work is to investigate on the role of microplastics and acid rain together, on *Lepidium sativum*, in controlled condition on different time of exposure. To carry out our experiment we used different kinds of microplastics: Polypropylene (PP), Polyethylene (PE), Polyvinylchloride (PVC), and a commercial mixture (PE+PVC). We have evaluated both at shoot and root level, in short and long time of exposure: i) biometric parameters (percentage inhibition of seed germination, relative growth rate of plant height, relative growth rate of leaf number and fresh biomass production), ii) oxidative stress (hydrogen peroxide, lipidic peroxidation, glutathione, ascorbic acid). While on plant exposed to long time, we have evaluated also: chlorophylls, carotenoids, aminolaevulinic acid, and soluble sugar production. We hypothesized that when *L. sativum* is treated with several types of microplastic and acid rain together in different time of exposure, short and long respectively, it will respond differently about its growth and physiological mechanisms undergoing to the first toxicity response.

Materials and Methods

Growth condition, experimental set up and biometrical traits

Certified seeds of *Lepidium sativum* were obtained from ECOTOX LDS. The Phytotox-kit from MicroBio Test Inc. were used for acute toxicity test with some modification. In brief, one plate supplied from Phytotoxkit, for each kind of microplastic tested, containing 10 seeds and filled with 90mL of commercial soil, without filter between roots and soil, was used. Before to sow seeds, the capacity field was tested on previously oven-dried soil, and then soil was soaked with 55mL of Milli-Q water or 55mL of acid rain; after that microplastics were added. A solution of acid rain, with a pH 4.5, was prepared as Liu et al. [19], by using H_2SO_4 and HNO_3. Acute stress was tested at 6 days from seeding.

As regard as chronic toxicity experiment, seeds were sown in pot (5.5cm diameter x 6cm depth) containing 50mL of commercial soil already dried, with the capacity field assessed, and microplastics supplied. One seed per pot and 10 seeds per treatment were sown; plants were regularly monitored and watered, twice a week with 8.5mL of Milli-Q water, or acid rain solution, per time, and sampled after 30 days from sowing. In both toxicity tests, plants were grown in a climatic chamber under controlled environmental conditions (temperature ranging between 20°C and 17°C; relative air humidity ranging between 40% and 60%; Photosynthetic Photon Flux Density (PPFD) of 700μmol$m^{-2}s^{-1}$ for 14h per day (from 06:00 to 20:00 local time). All the experiment was carried out by applying six different microplastic treatment conditions: i) control (C-) only commercial soil and MilliQ water, ii) control (C+) only commercial soil and watered with acid rain, iii) soil added with polyethylene and watered with acid rain (PE+), iv) soil added with polyvinylchloride and acid rain (PVC+), v) soil added with polypropylene plus acid rain solution (PP+), and vi) soil added with a commercial mixture of polyethylene/ polyvinylchloride and acid rain (PE+PVC+). The composition of the plastics used was confirmed by a Fourier Transformed Infrared spectrometer (FT-IR; Nicolet iN10; Thermo) prior to use.

In this study, we used 0.02% (w/w) content of microplastic, a lesser concentration than those found by Fuller and Gautman [20] in an industrial area. For each treatment about 0.092g of microplastic were added to 500mL (500g of soaked soil) of commercial soil. Microplastics fragments were obtained by grinding, with liquid nitrogen, of different pieces of macroplastic, obtained powder was then sieved by 0.125mm ASTM sieve to remove larger plastic fragments. The powder passing the sieve was collected and washed with alcohol and subsequently dried in oven at 40°C. Plant height measured by precision calliper, and leaf number were carried out once per week, from the start to the end of the experiments. The relative growth rate of shoot height (RGRh) and leaf number (RGRl) are calculated by the following formulas [10]:

$$RGR_h = ln\ H_2 - ln\ H_1/t_2 - t_1$$

$$RGR_l = ln\ L_2 - ln\ L_1/t_2 - t_1$$

where t_1 and t_2 are the times of the first and last measurements, respectively, carried out. H_1 and L_1 are the initial shoot height (cm) and leaf number (#l); while H_2 and L_2 are the final shoot height (cm) and leaf number (#l).

The germination rate were measured after 6 days from the begin of the experiment; percentage inhibition of seed germination was carried out with following formula [21]:

$$I\% = \frac{C_s - T_s}{C_s} \times 100$$

where C_s are the germinated seeds of control group, and T_s are the germinated seeds of each treatment. The germinated seed numbers are obtained from the average of the replicates used. The biomass was measured at the end of the experiment, during the sampling, by weighing shoot and root fresh weight.

Hydrogen peroxide, malondialdehyde, antioxidants, and soluble sugar determination

Hydrogen peroxide (H_2O_2) was measured spectrophotometrically after reaction with potassium iodide (KI), according to a method proposed by Alexieva et al. [22]. The reaction was developed in Trichloroacetic Acid (TCA) and absorbance measured at 390nm. The amount for H_2O_2 was calculated using standard curve prepared with known concentrations of H_2O_2. The results were expressed as μg^*g^{-1} fresh leaf weight (flw). The Malondialdehyde (MDA) concentration was measured by Thiobarbituric Acid (TBA) method [23], the concentration was expressed in μmol^*g^{-1} and calculated by following formula: C (μmol^*l^{-1}) = 6.45("A532"-A"600")-0.56A"450". Ascorbic acid (AsA) concentration was determined through the method proposed by Okamura [24] and modified by Law et al. [25]. The assay was based on the reduction of Fe3+ to Fe^{2+} by Ascorbate (As) in acidic solution. The absorbance at 525nm was recorded. A standard curve of Ascorbic Acid (AsA) was used for calibration. Results were expressed as μg^*g^{-1} flw. Glutathione (GSH) was determined using a modification of the Sedlak and Lindsay [26] method. The determination was obtained through the extraction in TCA and reaction with Ellman's reagent; the absorbance was read at 412nm. A standard curve of GSH was used for calibration. The results were expressed as μg^*g^{-1} flw. The soluble sugar extraction and determination was performed according to Dey [27], the absorbance at 485nm was recorded and the concentration was calculated by the use of the standard curve obtained with glucose; the results were expressed in mg^*g^{-1}. All spectrophotometric analyses were performed by UV/V is spectrophotometry (ONDA, mod. UV-30 Scan).

Pigments and aminolaevulinic acid determination

About 0.3g of fresh leaf sample was homogenized with 6mL of 80% acetone; then, sample mixture was centrifuged at 12,000rpm for 20min at 4°C. The supernatants were used to determine the chlorophylls and carotenoids content. Chlorophylls and carotenoids content were estimated by measuring the absorbance at 470, 645, and 663 nm. Then, chlorophyll-a, chlorophyll-b, total chlorophylls and carotenoids were further calculated according to formulae described by the literature [28]. The Aminolaevulinic Acid (AlA) leaf content was measured according to Haren and Klein [29]. The determination was obtained through the extraction in TCA and reaction with Ehrlich's reagent; the absorbance was read at 553nm. A standard curve of AlA was used for calibration. The results were expressed as μg^*g^{-1} fresh leaf weight. All spectrophotometric analyses were performed by UV/Vis spectrophotometry (ONDA, mod. UV-30 Scan).

Statistical analysis

Descriptive statistics (means, standard errors) were performed for all measured parameters using SigmaPlot 12.5 (SPSS Inc., Chicago, IL) scientific data analysis and graphing software. Analysis of variance, one-way ANOVA, was applied to test the different microplastics and acid rain effects on *Lepidium sativum* plants. A Fisher-LSD post-hoc test was applied to asses significantly differences among treatments ($p < 0.05$ level). Multivariate statistics were performed by Primer v7.0 (Primer-E Ltd., Plymouth Marine Laboratory, UK) on Euclidean matrices of distance calculated on normalized biometrical and physiological responses; this latter both at shoot than at root level, to evaluate the significance of observed segregations according to the factors of the time of exposure (two levels, long and short times), and different microplastics (PE, PVC, PE+PVC and PP). The AlA and pigments data are being evaluated for treatments (two levels, - MilliQ, + acid rain), and the different microplastics supplied during chronic exposure at foliar level.

Results

Effects on plant growth

In the acute stress, biometric traits analysed on garden cress are almost all statistically significant (Table 1). Results from ANOVA show a significant interaction effect between treatments and each biometrical parameter for I% ($p < 0.001$), RGRh ($p < 0.001$), RGRl ($p < 0.001$) and Br ($p=0.003$). Plants treated with PE+ and PVC+, separately, have shown the higher values for inhibition of germination, and lower values in terms of variations of height and for number of leaves emitted, always when compared to both controls. As regard biomass production, although not statistically significant, at shoot level, PE+ treated plants showed highest values then other treatments; while plants treated with PVC+ showed lower values than this latter. Regarding plants treated with commercial mixture, biomass production is lower than other treatments, while both negative and positive controls recorded higher values of this latter. An opposite scenario is emerged at root level, in term of biomass production; because highest values are shown by positive control while lower values are recorded for PE+ and PVC+ treated plants.

Table 1: Biometrical parameters obtained in *Lepidium sativum* plants exposed during acute toxicity experiments (6 days).

Acute Stress	I (%)		RGRh (cm)		RGRl (#l)		Bs (g)		Br (g)	
	mean	se	mean	se	mean	se	mean	se	mean	se
C-	0	0.000 (**d**)	0.057	0.005 (**b**)	0.208	0.023 (**a**)	0.047	0.008	0.014	0.001 (**b**)
C+	0	0.000 (**d**)	0.081	0.007 (**a**)	0.231	0.000 (**a**)	0.032	0.007	0.022	0.000 (**a**)
PE+	60.01	0.004 (**a**)	0.015	0.006 (**d**)	0.069	0.035 (**c**)	0.051	0.007	0.012	0.000 (**c**)
PVC+	30.041	0.011 (**b**)	0.046	0.012 (**c**)	0.139	0.037 (**b**)	0.035	0.002	0.012	0.000 (**c**)
PE+PVC+	0	0.000 (**d**)	0.081	0.008 (**a**)	0.231	0.000 (**a**)	0.025	0.005	0.015	0.002 (**b**)
PP+	10.029	0.008 (**c**)	0.048	0.009 (**c**)	0.116	0.038 (**b**)	0.035	0.008	0.014	0.001 (**b**)
***p*-level**	***		***		***		n.s		**	

Percentage of inhibition of germination (I%), relative growth rate of shoots height (RGRh cm), relative growth rate of leaf number (RGR #l), shoots biomass (Bs g) and root biomass (Br g) exposed to different microplastics are reported as mean values ± standard error (SE; n=10). One-way ANOVA was applied to determine significant differences between each treatment (p-level is given; *p <0.05; **p <0.01; ***p <0.001; ns: not significant). Different letters represent statistical differences between treatment for each chemical tested (Fisher-LSD multiple comparison, p <0.05 level).

Table 2: Biometrical parameters obtained in *Lepidium sativum* plants exposed during chronic toxicity experiments (30 days).

Chronic Stress	I (%)		RGRh (cm)		RGRl (#l)		Bs (g)		Br (g)	
	mean	se	mean	se	mean	se	mean	se	mean	se
C-	0	0.000 (**d**)	0.03	0.007 (**b**)	0.081	0.001 (**a**)	0.175	0.026 (**a**)	0.063	0.004 (**a**)
C+	0	0.000 (**d**)	0.042	0.004 (**a**)	0.063	0.001 (**b**)	0.08	0.008 (**c**)	0.028	0.007 (**b**)
PE+	30.01	0.004 (**b**)	0.015	0.004 (**b**)	0.037	0.008 (**c**)	0.094	0.004 (**c**)	0.037	0.004 (**b**)
PVC+	20.041	0,011 (**c**)	0.037	0.009 (**a**)	0.048	0.008 (**b**)	0.118	0.008 (**b**)	0.015	0.003 (**c**)
PE+PVC+	20.036	0,011 (**c**)	0.013	0.003 (**b**)	0.052	0.009 (**b**)	0.135	0.009 (**b**)	0.057	0.016 (**a**)
PP+	70.029	0.008 (**a**)	0.002	0.001 (**c**)	0.013	0.007 (**d**)	0.052	0.004 (**d**)	0.011	0.000 (**c**)
***p*-level**	***		***		***		***		**	

Percentage of inhibition of germination (I%), relative growth rate of shoots height (cm), relative growth rate of leaf number (#l), shoots biomass (Bs g) and root biomass (Br g) exposed to different microplastics are reported as mean values ± standard error (SE; n=10). One-way ANOVA was applied to determine significant differences between each treatment (p-level is given; *p <0.05; **p <0.01; ***p <0.001; ns: not significant). Different letters represent statistical differences between treatment for each chemical tested (Fisher-LSD multiple comparison, p <0.05 level).

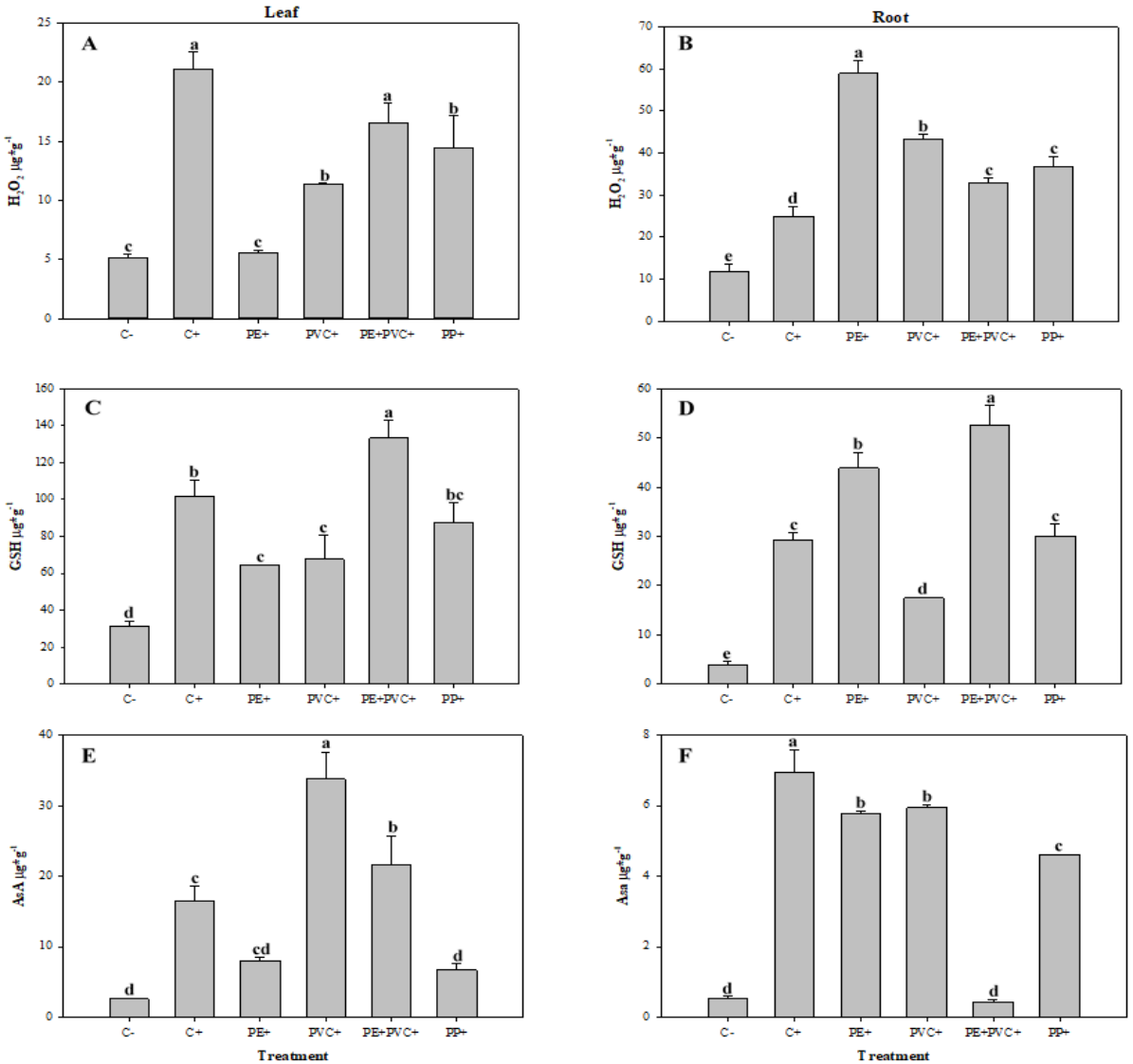

Figure 1: Biochemical responses to microplastic-induced stress obtained in *Lepidium sativum* plants exposed during acute toxicity experiments (6 days). Measured levels of Hydrogen peroxide (H_2O_2), ascorbic acid (AsA), and glutathione (GSH) in *L. sativum* leaves and roots treated with different microplastics are reported respectively in figure A, B, C, D, E and F. Data are expressed as mean ± standard error (SE, n=3). Different letters represent statistical differences between treatment for each chemical tested (Fisher-LSD multiple comparison, p <0.05 level).

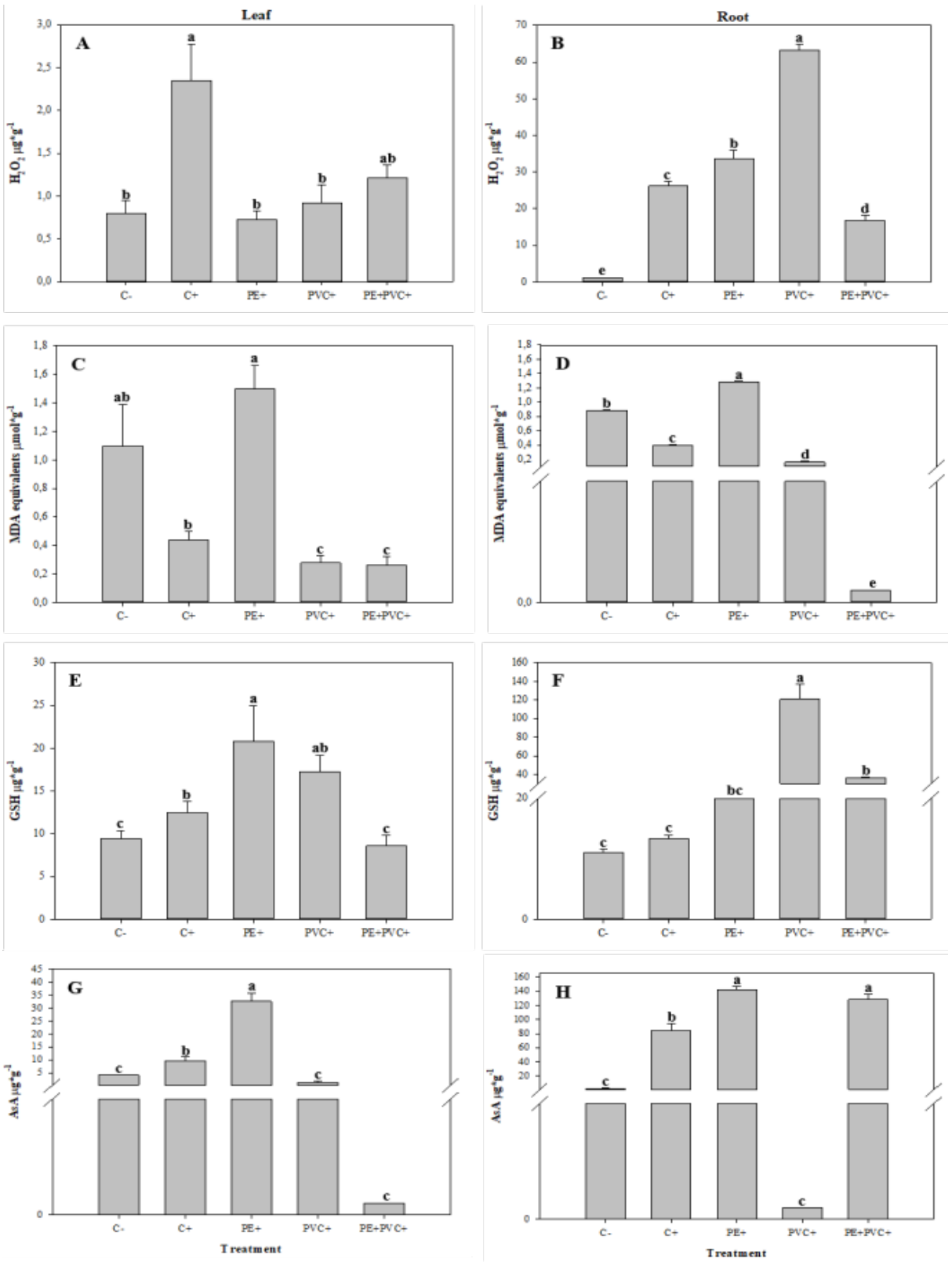

Figure 2: Biochemical responses to microplastic-induced stress obtained in *Lepidium sativum* plants exposed during chronic toxicity experiments (30 days). Measured levels of hydrogen peroxide (H_2O_2), Malondialdehyde (MDA), Ascorbic Acid (AsA), and Glutathione (GSH) in *L. sativum* leaves and roots treated with different microplastics are reported respectively in figure A, B, C, D, E, F, G and H. Data are expressed as mean ± standard error (SE, n=3). Different letters represent statistical differences between treatment for each chemical tested (Fisher-LSD multiple comparison, p <0.05 level).

The two controls plants and PE+PVC+ treated plants don't show any inhibition of germination; positive control plants and those treated with PE+PVC+ have the same values also for both height and leaf relative growth rate; while negative control plants exhibited slightly lower values. As regard PP+ treated plants, the biometrical traits seems to be not so much affected except for biomass production.

At the end of chronic stress, biometric traits of garden cress showed significant differences between treatments (Table 2), for each parameters taken in to account: I% ($p < 0.001$), RGRh ($p < 0.001$), RGRl ($p < 0.001$) Bs ($p < 0.001$) and Br ($p = 0.002$). The plants resulted most affected are those treated with PP+; they exhibited the highest inhibition of germination and lowest values for the other parameters respect to the other treatments. Such as in acute stress, also in chronic stress both controls plants don't have an inhibited germination; furthermore, negative control showed highest values then other treatments for relative growth rate of leaf and biomass production both at shoot than root levels, while positive control had highest values for relative growth rate of shoot height. The PVC+ and PE+PVC+ treatments exhibited almost the same values for each biometrical trait, except for the root biomass production that resulted almost 4-fold higher in this latter when compared with PE+ treated plants and the second values higher after negative control. Despite of PE+ treated plants had an inhibited germination at 30%, the other parameters don't appear so affected.

Effects of microplastics and acid rain on reactive oxygen species (ROS) and antioxidant system in *L. sativum*

In acute exposure, the compounds analysed, both in leaves and roots are statistically significant for each treatment. At foliar level, the ANOVA analyses show a significant interaction effect between each treatment and H_2O_2 ($p < 0.001$), GSH ($p < 0.001$) and AsA ($p < 0.001$) contents. The highest hydrogen peroxide (H_2O_2) and glutathione (GSH) concentrations are found in C+ and PE+PVC+ treated plants; while the plants that showed a highest ascorbic acid (AsA) production are those treated with PVC+ (Figure 1a, 1c and 1e). At root level, instead, we have a completely different scenario: the H_2O_2 production was always greater than foliar level, and plants most affected are those treated with PE+ and PVC+ respectively. The GSH and AsA productions are lower if compared with foliar level. The treatments that showed higher GSH production are PE+ and PE+PVC+, whilst higher AsA concentration is found in C+, PE+, and PVC+ treated plants (Figure 1b, 1d and 1f). Such as leaves, also in root the ANOVA analyses show a significant interaction effect between each treatment and H_2O_2 ($p < 0.001$), GSH ($p < 0.001$) and AsA ($p < 0.001$) contents.

During chronic exposure, plants treated with PP+ are almost all dead, and the few that remained are not numerically significant for each analysis carried out in this work, and for statistical analysis.

In chronic exposure, the ROS and antioxidants compounds, both in leaves and roots are statistically significant for each treatment.

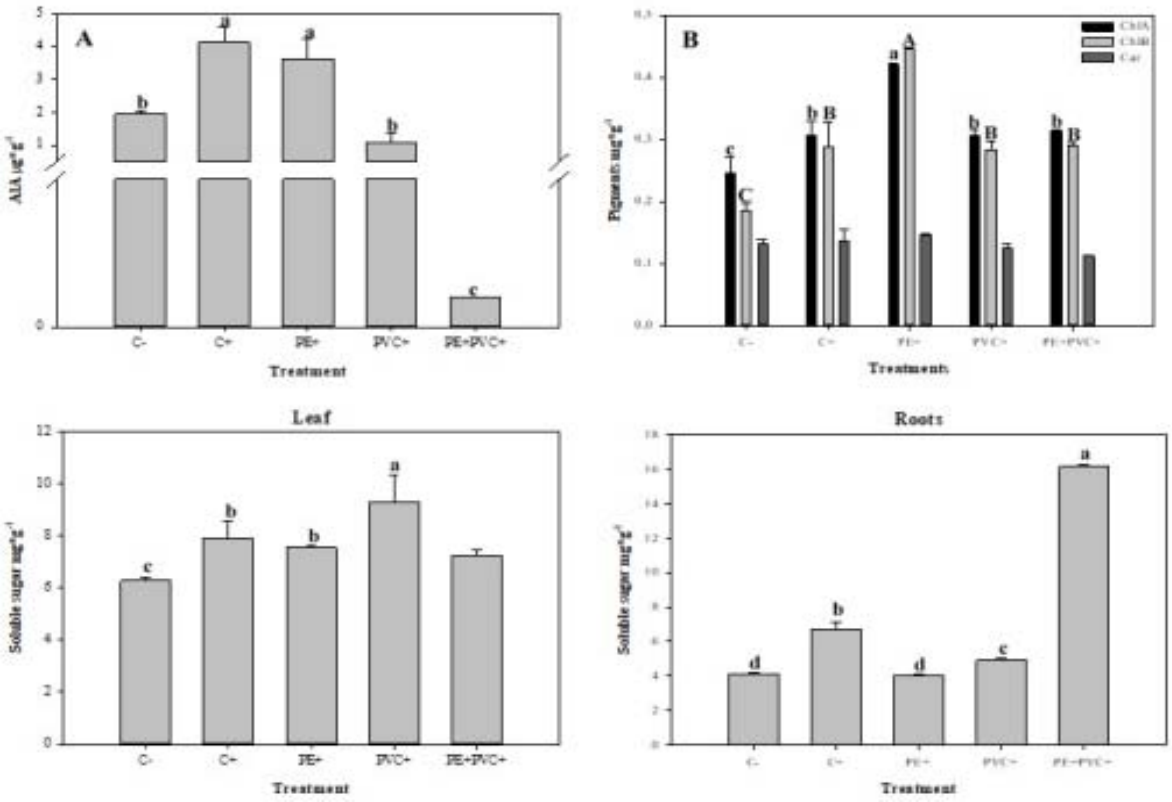

Figure 3: Aminolevulinic acid, pigments, and soluble sugar concentrations in *L. sativum* leaves and roots exposed to microplastic-induced stress during chronic toxicity experiments (30 days). Aminolevulinic Acid (AlA) is represented in Figure A, Chlorophylls (Chl-a, Chl-b) and carotenoids (Car) are represented in Figure B, while glucose in leaves and roots is represented in Figure C and D respectively. The values are expressed as mean ± standard error (SE, n=3). Different letters represent statistical differences between treatment for each compound (Fisher-LSD multiple comparison; $p < 0.05$ level).

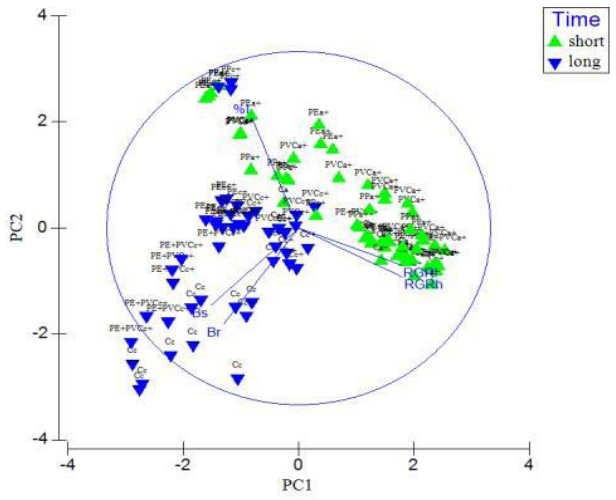

Figure 4: Principal component analyses performed on biometrical data. PCA performed on biometrical traits (I%, RGRh, RGRl and Bs and Br) of *L. sativum* exposed to different microplastics and acid rain (+) in long (c) and short (a) exposures.

In the ANOVA analyses carried out at foliar level, a significant interaction has been found for each treatment and H_2O_2 (p=0.006), MDA (p=0.013), GSH (p=0.019) and AsA (p <0.001) productions. The highest H_2O_2 production is observed in C+ treated plants; while PE+ treated plants showed highest content for malondialdehyde (MDA), GSH and AsA (Figure 2a, 2c, 2e and 2g). At root level, except for MDA, the other metabolites content is always higher than leaves (Figure 2b, 2d, 2f and 2h). The plants treated with PVC+ have shown highest production of H_2O_2, the MDA content has recorded almost the same trend of leaves; moreover, the treatment that recorded the highest MDA values is PE+. As regarding GSH values, they showed an increasing trend from C- to PVC+ treated plants, to then decrease in PE+PVC+ treated plants, such as for H_2O_2. Concerning to AsA content, higher production is recorded for PE and PE+PVC+ treated plants. Here The ANOVA analyses show a significant interaction between treatments and H_2O_2 (p <0.001), MDA (p <0.001), GSH (p <0.001) and AsA (p <0.001) productions.

Effects of microplastics and acid rain on aminolevulinic acid, photosynthetic pigments and soluble sugar in *L. sativum*

In chronic exposure, higher Aminolevulinic Acid (AlA) productions (Figure 3a), compared to the negative control (C-), are observed in C+ and PE+ treated plants; regarding as photosynthetic pigments (Figure 3b) higher concentration is showed to PE+ treated plants. At foliar level higher soluble sugar concentration (Figure 3c) is recorded for PVC+ treated plants, while at root level (Figure 3d) higher concentration are carry out for PE+PVC+ treated plants. The ANOVA analyses show that a significant interaction between treatment is found for AlA (p <0.001), Chl-a (p=0.006), Chl-b (p <0.001), glucose (p=0.019) at leaf level and glucose (p <0.001) at root level.

An overview on multivariate statistics

The Principal Component Analysis performed on biometrical traits (percentage inhibition of germination, relative growth rate of height and leaf, shoot and root biomass production) both at short than long exposure (Figure 4), explained 94.9% of the total variance (47.5, 31.9, 9.1 and 6.4% respectively). The eigenvectors related to PC1 have recorded a positive correlation for RGRh (0.539) and RGRl (0.535), while negative correlation were showed for I%, Bs and Br (-0.245, -0.458 and -0.392 respectively). Conversely, PC2 resulted positively correlated only for I% (0.625), the remaining eigenvectors were negatively correlated: RGRh (-0.276), RGRl (-0.209), Bs (-0.437) and Br (-0.545). The results of PERMANOVA analysis based on the Euclidean distance, performed on treatments versus time, has noticed 16.042 and 0.001 values for Pseudo-F and P respectively.

PCA performed on oxidant (H_2O_2) and antioxidants (GSH, AsA) compound at foliar level (Figure 5a), showed the 99.9% of total variance so divided: 70.1, 26.4 and 3.4% respectively. The eigenvectors related with PC1 were all positives and between these, showed higher correlation for H_2O_2 (0.647) and GSH (0.651); while for PC2 AsA was the only positive and strongly correlated (0.917) compared with other two compounds, H_2O_2 (-0.295) and GSH (-0.268). PERMANOVA analysis exhibited 28.774 and 0.001 values for Pseudo-F and P. PCA conducted on the roots (Figure 5b) has noticed a 99.9% of variance

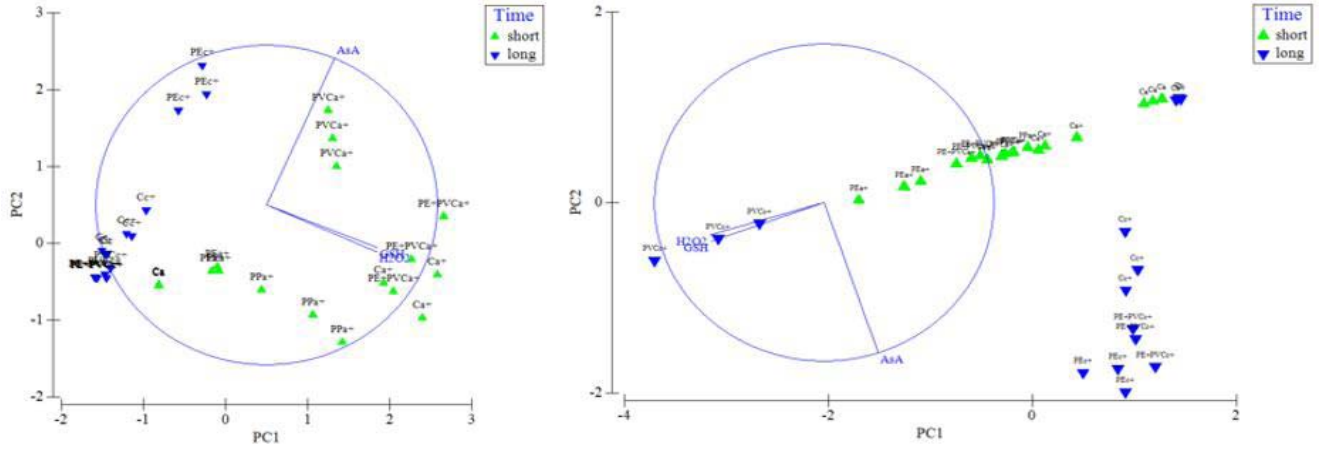

Figure 5: Principal component analyses performed on physiological data. PCA performed on oxidant (H_2O_2) and antioxidants (GSH, AsA) compounds of *L. sativum* (A) leaf, (B) roots exposed to different microplastics and acid rain (+) in long (c) and short (a) exposures.

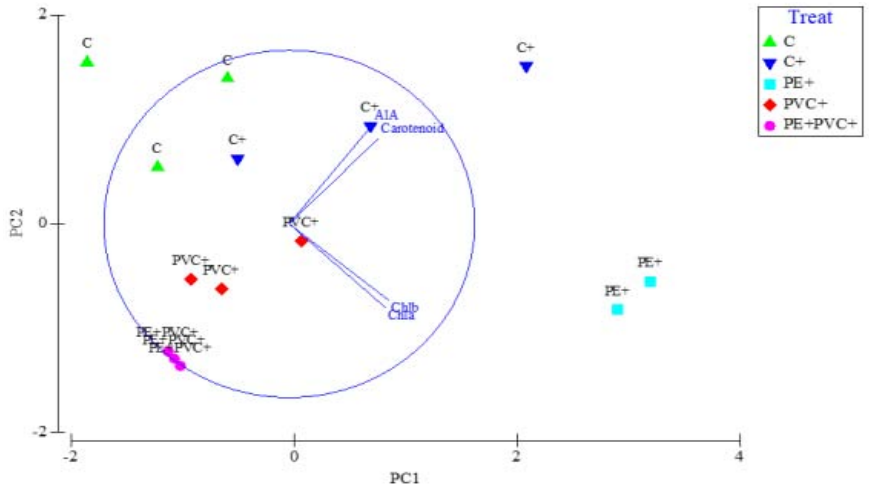

Figure 6: Principal component analyses performed on pigments. PCA performed on foliar AlA and pigments of *L. sativum* exposed to different microplastics and acid rain (+) during long time.

(58.5, 30.5, and 10.9%). The eigenvectors obtained from PC1 showed two negative correlations for H_2O_2 (-0.673) and GHS (-0.667); PC2 highlighted three negative correlations, but only AsA was strongly correlated (-0.947). Values recorded from PERMANOVA analysis were 66.776 and 0.001 for Pseudo-F and P separately. PCA carried out on AlA and pigments (Chl-a, -b, and carotenoids) showed 99.1% of the variance (64.9, 28.6, and 5.7%). The eigenvectors resulted from PC1 were all positively correlated, in major way Chl-a (0.524), Chl-b (0.540), and in minor way Car (0.482) and AlA (0.448). The eigenvectors obtained from PC2, were showing two negative correlations for Chl-a and -b (-0.486 and -0.433 individually), and other 2 positives for Car (0.493) and AlA (0.570); Figure 6. The PERMANOVA analysis, performed on treatments versus acid rain, has noticed 11.882 and 0.001 for Pseudo-F and P.

Discussion

Different types of microplastics and acid rain together are able to affect, differently, biometrical traits of garden cress. After short exposure (6 days), both of the control treatments, with (C+) and without acid rain (C-) showed similar values; except for relative growth rate of shoots height and root biomass that reported a higher value for plant treated with acid rain; this treatment has highlighted, also, a highest root biomass production then other treatments. These results indicate that, moderately acid rain don't affect negatively plant develop and growth. Concerning as plants treated with microplastics and acid rain together, polyethylene (PE+) treated plants resulted most affected for all the parameters except shoot biomass, that it showed the higher value. The lowest shoot biomass production is recorded for the commercial mixture (PE+PVC+) treatment, it has been the only trait negatively affected from this plastic, while the other traits showed values comparable to the control plants. After long exposure (30 days), the two control treatments show a differentiation for the biometrical traits carried out. Except for the germination, control plant treated with acid rain have reported lower values than untreated control plants for biomass and leaf number, conversely for shoot height they have higher value than C-. As regarding plants treated with microplastics and acid rain together, a further scenario is happened; polypropylene (PP) treated plants were the most negatively affected for each parameter considered. Noteworthy is that PVC+ and PE+PVC+ have shown almost comparable values and don't seem be so much negatively affected, except for root biomass in case of PVC+; while PE+ treated plants had lower germinability leaf number and shoot biomass than these latter two treatments. From a comparison carried out from these two different time of exposure, is clear that plants respond in different manner not only for the different treatments supplied, but also for the entire duration of the experimentation. At control level, is emerged that in long period plants cannot counteract negatively effects due to acid rain; also PP+ treated plants have reported a noticeable worsening in long exposure time; while, interestingly, plants treated with PE+ have shown an improvement in long period that can indicate a sort of acclimation to these conditions. The multivariate analysis based on Euclidean distance, was performed to analyses the separation between different types of microplastics in two times of exposure. The first principal coordinate axis showed that acute toxicity (short time) have an effect on different microplastics supply principally. Conversely, long time treated plants were separated along the second principal coordinate axis, with a trend shifted toward down side. From the results of this analysis, is clear that different times of exposure have a different impact on plant biometrical traits, confirming the differences in plant growth and development obtained from this study. Because the microplastics are considered new generation contaminants [1], in the literature there are few works that take into account plant-microplastics interaction in long time, and even more, the toxicity caused from microplastics associated to acid rain. Considering acute exposure, our results agree whit those obtained from Bosker [30] on garden cress, where they have found a decreased growth and development due to the plastic treatments, caused by the decreased germinability due to the plastic clogging at the seed test level. Concerning as acid rain, there are discordant results found on toxicity caused from it on the plants at biometrical level, mainly because it is pH values and plant species dependent. On tomato is emerged that when acidic pH values decrease, these had a negative effect on biometric traits [13]; but on the other hand, on *Elaeocarpus gravipetalus* and *Jatropha*

curcas [10,19] acid rain affect positively biometrical traits. Overall, our results for acute exposure are in agreement with those found on these latter two species, but concerning as chronic exposure our findings are in agreement only for shoots height that showed higher growth than other treatments; this is could be explained by the nitrogen, indirectly, added through acid rain application that could have enhanced the develop of plant growth [19].

Normally, plants produce Reactive Oxygen Species (ROS) during their life cycle; but when they are subjected to environmental stress this production is increased [31]. The hydrogen peroxide (H_2O_2) production is due to oxygen (O_2) consuming during respiration, this ROS cover a dualistic function: at low concentration it works such as signaling molecule, but at high concentration it can cause cell dead, that which in turn bring to oxidative burst [32]. In a recent study, it was demonstrated that microplastics can induce oxidative stress through H_2O_2 production [33]; but what happen if to the MPs is added acid rain also? Can the toxicity of each microplastic remain unchanged or change between these? Furthermore, what happen at roots level? Our results on H_2O_2 content highlight a higher production during the short exposure both at shoot than at root level; and at root level, in particular we have recorded always a higher production than shoot. From these results, seem that at shoot level the toxicity is more affect to the acid rain supplied that than different microplastics used, however the plants treated with PE+PVC+ together were most negatively affected than other treatments. Noteworthy is that both C+ than PE+PVC+ treated plants have shown a similar trend at biometrical level, indicating that these two treatments had a negative effect only for biomass production; this can be explained by the fact that plants were more involved in counteracting oxidative stress than in the biomass production. Conversely, a totally opposite different scenario appear for roots, here is clear that oxidative stress is give from different MPs supplied, and particularly affected were plants treated with PE+ followed to those treated with PVC+. In this case the acid rain coupled with MPs had a negative effect for all the biometrical parameters analyzed, except for shoot biomass production because it resulted higher than C+ and PE+PVC+ treatments respectively. This can be explained by the fact that plants that are able to survive and counteract toxically effects due to the treatments supplied, spend their resource in biomass production rather than growth or leaf emission.

In the long exposure, from the results obtained to H_2O_2 production, is emerged that roots are more affected than shoots. At shoot level, our results show a similar trend of short exposure, although the recorded values are about 10-fold lower. The C+ treated plants have shown the higher values, followed to PE+PVC+ treated plants. Interestingly, at root level, plant treated with PVC+, resulted more affected, followed to PE+ treated plants. In long exposure, these latter two treatments showed an opposite trend compared to short exposure, almost to indicate that in long period PVC+ became more toxic, or that plant is not able to counteract its toxic effect, compared with PE+. Such as H_2O_2, also Malondialdehyde (MDA) is considered an indicator to determine the oxidative stress degree in plants [34], it is a toxically compound at cytological level, derived from lipidic peroxidation [35]. Our results, highlight that the MDA production both at shoot and root level, is almost the same, and the plants most affected were those treated with PE+.

In order to counteract oxidative stress and so avoid cellular dead, plants have developed an antioxidant system. The main players of this system are ascorbate and glutathione (GSH) that with their ascorbate/glutathione cycle, representing the main detoxifying system [32]. GSH is a low molecular weight thiolic antioxidant, it is involved in several physiological processes such as in defence against ROS by reducing superoxide, and particularly, in H_2O_2 metabolism it works to produce ascorbate from Dehydroascorbate (DHA) both chemically or enzymatically [36,37]. Overall, in the short exposure, our results have recorded a higher production at shoot than root level. At foliar level, plants treated with PE+PVC+, followed to C+, have shown a higher GSH content such as for the H_2O_2 production. At radical level, the main producers of GSH were PE+PVC+ and PE+ treated plants, these results can have a dual explanation: this high concentration can be due both to counteracting action against ROS, but also to generating ascorbate from Haliwell-Asada cycle. In the long exposure, GSH concentrations are mostly lower than short exposure, either at shoot or at root level; except for the roots of PVC+ treated plants that showed the highest GSH production. From a more in depth analyses, it emerged that at shoot level PE+ treated plants have shown the higher GSH concentration such as for MDA production. Noticeable is also the GSH produced from PVC+ treated plants. At radical level, the highest GSH production come from plants treated with PVC+, such as happened for H_2O_2 concentration; also this production can have worked in double way as explained above for the short exposure. In a previous study conducted only on microplastics [33], the GSH values obtained were overall lower than those obtained here, this can be explained by the fact that sulphate ion (SO_4^{2-}) supplied indirectly by acid rain could be assimilated and used by plants to produce GSH [38].

The Ascorbic Acid (AsA), in plants, cover an important multiple functions, as mentioned above for its involvement in ascorbate/glutathione pathway, and also both as cofactor for peroxidases and for its ability to directly remove ROS [37]. Overall, at foliar level AsA production is higher in the short that in long exposure, while the highest production is recorded at root level in long exposure. During the short exposure, at foliar level, the higher AsA production is shown by PVC+ followed to PE+PVC+ treated plants. Noteworthy, in this exposure, is that at radical level are been recorded the lowest AsA contents; and the treatments that have shown higher productions are C+ followed to PE+ and PVC+ treated plants, these two latter in the same way. In the long exposure, PE+ treated plants have shown the higher AsA production in shoot; this result is in agreement with those found for GSH and lipidic peroxidation. From the values obtained from this two antioxidants, is clear that they have worked together to counteract lipid peroxidation caused by polyethylene supplied with acid rain. A different scenario is showed at root level; higher productions are recorded for PE+ and PE+PVC+ treated plants. Also in this case is clear that these two antioxidants have co-worked together to try to contrast dangerous effect due to lipidic peroxidation and H_2O_2. Interestingly, is that AsA production from PVC+ treated plants is resulted lowest then other treatments; this result is completely opposite to those obtained by GSH production. In this case, the AsA depletion to counteract chronic toxicity was replaced by GSH production; a similar trend was recorded in the short exposure always at radical level for PE+PVC+ treated plants. Globally, our results on oxidative burst indicate that during the short exposure plant more affected at shoot level were C+ and PE+PVC+ treated plants, while

the same plants, at radical level resulted less affected. Conversely, at root level, the plants more affected, PE+ and PVC+ respectively, at shoot level resulted less affected. In long exposure, the same scenario is happened, plant more affected at shoot level were less affected at radical level and vice-versa. However, in the long exposure, at shoot level seem that plants are able to adapt them to microplastics and acid rain toxicity, it is supported by the low concentration of H_2O_2 produced, while at root level, the toxicity are shifted toward PVC+ treated plants and for the others treatments the ROS concentration were generally lower than short exposure, this can indicate that plants could be counteract in long time the stress induced by the microplastics and acid rain supplied; although other analyses are needed to investigate in depth on this topic. Such as for the results mentioned above, that have highlighted a completely different trend between short and long time of exposure both at foliar than root level, also multivariate analysis has confirmed them. At shoot level, treatments during the short exposure are separated, mostly, along the PC1 except PVC+; while at the contrary, in the long time treatments are separated along PC2. At root level, plant subjected to the short time treatments are separated exclusively along PC1; while on the other hand long time treated plants are separated mainly on PC2. Overall, our results indicate that different time of exposures exert a different effect on the treatments.

When plants are subjected to stressful condition the photosynthetic machinery could be negatively affected, this can be reflected on pigments biosynthesis and at entire level of tetrapyrroles pathway [39]. Concerning as chlorophyll and carotenoid productions, in the long exposure, our treatments don't seem affect negatively their production; although there are different concentration of pigments respect to the different treatments supplied, except for carotenoids content that don't show any differences between treatments. Compared with our previous work [33], where plants were supplied only with microplastics, here pigments values are lower overall, and in particular as regards chlorophyll a (Chl-a) that shows closer values to Chl-b. This unconventional Chl-b production can be attributable to the fact that this pigment is formed from the Chl-a. Normally, Chl-a concentration is higher than Chl-b because it is present both in photosynthetic reaction centers and in light-harvesting antennae [40]. Our results suggest that acid rain could probably cause an impairment in Chl-a production, that which in turn results in a lower production of this latter pigment, or that the demand of Chl-b is higher respect to the Chl-a production.

Higher pigments contents are shown by PE+ treated plants, it's interesting to note that for this treatment we have obtained one of the lower values concerning as biomass production and germination rate, same results were obtained from our previous work [33] and on *Triticum aestivum* [41]. Based on the results obtained from our research we can postulate that plants that were able to germinate and grow spend their resource in favour of photosynthetic activity than biomass production. Aminolevulinic acid (AlA) is involved not only in chlorophyll synthesis, but also in stress tolerance by inducing antioxidant compounds such as glutathione and ascorbic acid [42]. In this study, globally, AlA production was lower than our previous work that involved the use of microplastics alone; this result can be explained by the addition of the acid rain that can have interfered with its production. However, the treatments that showed higher AlA production were C+ and PE+, these findings are in agreement with pigments production, but can have another explanation also. As mentioned above, AlA is involved in counteracting oxidative burst, from data collected here, at shoot level, plants more affected were those treated with C+ and PE+ as shown by higher content of H_2O_2 and MDA respectively; moreover, the same treatments have reported a higher production of glutathione and ascorbic acid. Regarding as multivariate analysis is evident that different treatments supplied under acid rain condition exert a different effect on plants.

Sugars are involved in many physiological roles, but before of these they are considered the main carbon and energy source. At physiological level, instead, they cope some important functions in growth and development, metabolism and stress resistance [43]. Concerning this latter feature, they have an osmoregulation action, in particular, during drought and salt stresses [44]. Our results show that apparently, microplastic plus acid rain treatments did not affect, negatively, soluble sugar concentrations at shoot level. Plants that recorded higher sugar concentration were those treated PVC+, and interestingly, the same treatment shown higher GSH production; this latter result is been found also on tomato and wheat, subjected with other kind of stresses [45,46]. Results collected at radical level, showed generally lower soluble sugar concentrations than shoot; except for PE+PVC+ treated plants that recorded higher values than all at radical level, about 10 fold higher, but also at shoot level; highlighting that stress sensing at root level is different than at shoot level.

Conclusion

Our experiment demonstrates that different kinds of microplastics supplied with acid rain are able to interfere in different ways both in short and in long exposure, but also at shoot and root levels, on garden cress. Furthermore, microplastics and acid rain together are able to affect negatively germination, growth and development such as for PE+ treated plants in short exposure; and PP+ treated plants in long exposure. At physiological level, our treatments were able to induce oxidative burst and the related antioxidants responses, both at shoot then at root level. H_2O_2 is always higher in short exposure and at radical level, GSH is mostly higher in short exposure at shoot level, except for PVC+ treated plants in long exposure in roots; and also higher AsA values, are recorded in this latter exposure, at radical level. Concerning as toxicity induced by our treatments, the shoots were most affected by acid rain, while root were most affected by different microplastics used coupled with acid rain. In particular, in acute toxicity, at radical level PE+ resulted the most stressful treatments, meanwhile in chronic exposure the toxicity is shifted toward PVC+ treatments although PE+ remains also toxic as confirmed by result collected from MDA analyses. Always in long exposure the toxicity of PE+ and PVC+ are been confirmed by aminolevulinic acid and soluble sugar analyses, respectively.

Highlights

- *L. sativum* was exposed to different microplastics coupled with acid rain.
- Roots are more affected to microplastics and acid rain then shoots.
- In long exposure, plants treated with polypropylene were not able to growth and develop.

- Plants, in long time, were not able to counteract PVC negative effects.

- PVC treated plants in short and long exposure, at shoot level, keep the same values of H_2O_2 and GSH.

Declarations

Authors' contribution: S.P: Performed experiment development, statistical analyses, and the first, paper draft; A.B: Performed laboratory analyses; M.R: Perform found recruitment, experimental project plan, revision of the first draft for the production of the submitted version.

Funding: This research was funded by Bioscience Research Center, grant number 042019BSRC, research grant holder Monia Renzi.

Ethics approval: Authors declare the ethical compliance of the research to ethic guidelines for scientific researches.

References

1. Qi R, Jones DL, Li Z, Liu Q, Yan C. Behavior of microplastics and plastic film residues in the soil environment: A critical review. Sci. Total. Environ. 2020; 703: 134722-134733.
2. De Souza Machado AA, Kloas W, Zarfl C, Hempel S, Rillig MC. Microplastics as an emerging threat to terrestrial ecosystems. Glob Change Biol. 2018; 24: 1405-1416.
3. Plastics Europe. Plastics - the facts 2018: an analysis of European plastics production, demand and waste data. 2018.
4. Duis K, Coors A. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ. Sci. Eur. 2016; 28: 1-25.
5. Wang W, Ge J, Yu X, Li H. Environmental fate and impacts of microplastics in soil ecosystems: Progress and perspective. Sci. Total Environ. 2020; 708: 134841-134850.
6. Horton AA, Walton A, Spurgeon DJ, Lahive E, Svendsen C. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci. Total Environ. 2017; 586: 127-141.
7. Rillig MC, Ingraffia R, de Souza Machado AADS. Microplastic incorporation into soil in agroecosystems. Front. Plant Sci. 2017; 8: 1805.
8. Yadav V, Arif N, Kovac J, Pratap Singh V, Kumar Tripathi D, Kumar Chauhan D, et al. Structural modifications of plant organs and tissues by metals and metalloids in the environment: A review. Plant Physiol. Biochem. 2021; 159: 100-112.
9. Wu Y, Liu H, Wang Q, Zhang G. Roles of cytokinins in root growth and abiotic stress response of *Arabidopsis thaliana*. Plant Growth Regul. 2021; 94: 151-160.
10. Shu X, Zhang K, Zhang Q, Wang W. Ecophysiological responses of *Jatropha curcas L*. seedlings to simulated acid rain under different soil types. Ecotoxicol. Environ. Saf. 2019; 185: 109705.
11. Evans LS. Botanical aspects of acidic precipitation. Bot Rev. 1984; 50: 449-490.
12. Xu HQ, Zhang JE, Ouyang Y, Lin L, Quan GM, Zhao BL, et al. Effects of simulated acid rain on microbial characteristics in a lateritic red soil. Environ. Sci. Pollut. Res. 2015; 22: 18260-18266.
13. Debnath B, Irshad M, Mitra S, Li M, Rizwan HM, Liu S, et al. Acid rain deposition modulates photosynthesis, enzymatic and non-enzymatic antioxidant activities in tomato. Int. J. Environ. Res. 2018; 12: 203-214.
14. Isah T. Stress and defense responses in plant secondary metabolites production. Biol. Res. 2019; 52: 39.
15. Choudury S, Panda P, Sahoo L, Panda SK. Reactive oxygen species signaling in plants under abiotic stress. Plant Signal. Behav. 2013; 8: e23681.
16. Nehdi IA, Sbihi H, Tan CP, Al-Resayes SI. Garden cress (*Lepidium sativum Linn*.) seed oil as a potential feedstock for biodiesel production. Bioresour. Technol. 2012; 126: 193-197.
17. Janecka B, Fijalkowski K. Using *Lepidium sativum* as a test phytotoxicity from lead/zinc spoils and soil conditioners. Soil chemical pollution, risk assessment, remediation and security: part of the series NATO science for peace and security. Springer-Verlag, Dordrecht. 2008: 177-182.
18. Smolinska B, Leszczynska J. Photosynthetic pigments and peroxidase activity of *Lepidium sativum L*. during assisted Hg phytoextraction. Environ. Sci Pollut. Res. 2017; 24: 13384-13393.
19. Liu M, Korpelainen H, Dong L, Yi L. Physiological responses of *Elaeocarpus glabripetalus* seedlings exposed to simulated acid rain and cadmium. Ecotoxicol. Environ. Saf. 2019; 75: 118-127.
20. Fuller S, Gautam A. A procedure for measuring microplastics using pressurized fluid extraction. Environ. Sci. Technol. 2016; 50: e5774-e5780.
21. ISO. Soil quality-determination of the toxic effects of pollutants on germination and early growth of higher plants. 2016; 18763.
22. Alexieva V, Sergiev I, Mapelli S, Karanov E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ. 2001; 24: 1337-1344.
23. Heath RL and Packer L. Photoperoxidation in isolated chloroplasts: i. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 1968; 125: 189-198.
24. Okamura M. An improved method for determination of L-ascorbic acid and L-dehydroascorbic acid in blood plasma. Clin. Chim. Acta. 1980; 103: 259-268.
25. Law MY, Charles SA, Halliwell B. Glutathione and ascorbic acid in spinach (*Spinacea oleracea*) chloroplasts. The effect of hydrogen peroxide and of Paraquat. J Biochem. 1983; 210: 899-903.
26. Sedlak J, Lindsay RH. Estimation of total, protein-bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968; 25: 192-205.
27. Dey P. Oligosaccharides. In Methods in plant biochemistry. 1990; 2: 189-218.
28. Bhushan D, Pandey A, Choudhary MK, Datta A, Chakraborty S & Chakraborty N. Comparative proteomics analysis of differentially expressed proteins in chickpea extracellular matrix during dehydration stress. Molecular & Cellular Proteomics. 2007; 6: 1868-1884.
29. Harel E and Klein S. Light dependent formation of 5-aminolevulinic acid in etiolated leaves of higher plants. Biochem. Biophy. Res. Commun. 1972; 49: 364-370.
30. Bosker T, Bouwman LJ, Brun NR, Behrens P, Vijver, MG. Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant Lepidium sativum. Chemosphere. 2019; 226: 774-781.
31. Ahmad P, Alyemeni MN, Ahanger MA, Wijaya L, Alam P, Kumar A, et al. Upregulation of antioxidant and glyoxalase systems mitigates NaCl stress in *Brassica juncea* by supplementation of zinc and calcium. J. Plant Interact. 2018; 13: 1510162.
32. Quan LJ, Zhang B, Shi WW, Li HY. Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. J. Integr. Plant. Biol. 2008; 50: 2-18.
33. Pignattelli S, Broccoli A, Renzi M. Physiological responses of garden cress (*L. sativum*) to different types of microplastics. Sci. Total Environ. 2020; 727: 138609.
34. Lin Q, Zhou C, Chen L, Li Y, Huang X, Wang S, et al. Accumulation and associated phytotoxicity of novel chlorinated polyfluorinated ether sulfonate in wheat seedlings. Chemosphere. 2020; 249: 126447.
35. Zhang J, Li X, Zhou L, Wang L, Zhou Q, Huang X. Analysis of effects of a

new environmental pollutant, bisphenol A, on antioxidant systems in soybean roots at different growth stages. Sci. Rep. 2016; 6: 23782.

36. Cheng MC, Ko K, Chang WL, Ko WC, Chen GH, Lin TP. Increased glutathione contributes to stress tolerance and global translational changes in Arabidopsis. Plant J. 2015; 83: 926-939.

37. Noctor G, Reichheld JP, Foyer CH. ROS-related redox regulation and signaling in plants. Semin. Cell Dev. Biol. 2018; 80: 3-12.

38. Qiao F, Zhang XM, Liu X, Chen J, Hu WJ, Liu TW, et al. Elevated nitrogen metabolism and nitric oxide production are involved in Arabidopsis resistance to acid rain. Plant. Physiol. Biochem. 2018; 127: 238-247.

39. Dalal VK, Tripathy BC. Modulation of chlorophyll biosynthesis by water stress in rice seedlings during chloroplast biogenesis. Plant, Cell and Environment. 2012; 9: 1685-1703.

40. Fang L, Ma Z, Wang Q, Nian H, Ma Q, Huang Q, Mu Y. Plant growth and photosynthetic characteristics of soybean seedlings under different LED lighting quality conditions. J. Plant Growth Regul. 2020.

41. Qi YL, Yang XM, Pelaez AM, Lwanga EH, Beriot N, Gertsen H, et al. Macro- and micro- plastics in soil-plant system: effects of plastic mulch film residues on wheat (*Triticum aestivum*) growth. Sci. Total Environ. 2018; 645: 1048-1056.

42. Wu WW, He SS, An YY, Cao RX, Sun YP, Tang Q, et al. Hydrogen peroxide as a mediator of 5-aminolevulinic acid-induced Na+ retention in roots for improving salt tolerance of strawberries. Physiol. Plant. 2019; 167: 5-20.

43. Rolland F, E Baena-Gonzalez and J Sheen. Sugar sensing and signaling in plants: conserved and novel mechanisms. Ann. Rev. Plant Biol. 2006; 57: 675-709.

44. Patade VY, Bhargava S, Suprasanna P. Salt and drought tolerance of sugarcane under iso-osmotic salt and water stress: growth, osmolytes accumulation, and antioxidant defense. J Plant Interact. 2011; 6: 275-782.

45. Rady MM, Belal HEE, Gadallah FM, Semida WM. Selenium application in two methods promotes drought tolerance in *Solanum lycopersicum* plant by inducing the antioxidant defense system. Sci. Hortic. 2020; 266: 109290.

46. Alzahrani Y, Rady MM. Compared to antioxidants and polyamines, the role of maize grain-derived organic biostimulants in improving cadmium tolerance in wheat plants. Ecotox. Environ. Safe. 2019; 182: 109378.