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Phytotoxicity of wear debris from traditional and innovative brake pads

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ABSTRACT

Traffic-related emissions include gas and particles that can alter air quality and affect human and environmental health. Limited studies have demonstrated that particulate debris thrown off from brakes are toxic to higher plants. The acute phytotoxicity of brake pad wear debris (BPWD) investigated using cress seeds grown in soil contaminated with increasing concentrations of debris. Two types of pads were used: a commercially available phenol based pad and an innovative cement-based pad developed within of the LIFE+ COBRA project. The results suggested that even through the BPWD generated by the two pads were similar in and morphology, debris from traditional pads were more phytotoxic than that from cementitious pads, causing significant alterations in terms of root elongation and loss of plasma membrane integrity.

1. Introduction

Road traffic is certainly one of the anthropogenic sources with most impact on air quality: exhaust and non-exhaustive emissions from vehicles are the main contributors to environmental particulate matter concentrations [van der Gon et al., 2013]. Traffic-related emissions of gas and particles are putting increasing pressure on human and environmental health, in particular in urban areas [Borrego et al., 2000; Host et al., 2012; Smith et al., 2017]. While the impact of gaseous pollutants and particulate matter from combustion emissions is has been widely studied, less attention has been dedicated to non-exhaust emissions such as tire and brake pad debris [Gualtieri et al., 2005a; Gualtieri et al., 2005b; Kukutschova et al., 2009; Shupert et al., 2013; Dodd et al., 2014; Villena et al., 2017]. In fact, only three studies have assessed the impact of wear debris from brake pads on ecological receptors, highlighting potential toxic effects of brake pad wear debris (BPWD) on bacteria, aquatic and terrestrial plants [Kukutschova et al., 2009; Shupert et al., 2013; Dodd et al., 2014].

We investigated the acute phytotoxicity of brake pads wear debris (BPWD) using cress (*Lepidium sativum*) seeds as a model of dicotyledon. Cress is one of the most sensitive species used in phytotoxicity tests, especially regarding metal toxicology (APAT, 2004; Baderna et al., 2015a, 2015b). We used a standardized assay based on seed germination and root elongation coupled with Evans blue assay in order to evaluate the root damage of seedlings grown in soils contaminated with BPWD. Two brake pads were used to generate BPWD: a traditional phenol-based pad available on the market and an innovative cementitious pad developed within the LIFE+ COBRA Project [LIFE13 ENV/IT/000492]. The innovative brake pads were formulated to reduce the impact in terms of global warming potential (GWP) of the raw materials and production process and to reduce the emission of organic compounds during braking. Indeed, organic components as rubbers, aramid fibers and cashew friction dust, used in state-of-the-art brake pads have been removed in cementitious brake pads. Moreover, thermoset traditional binders as phenolic resins have been substituted in cementitious brake pads by hydraulic inorganic binders, allowing to avoid any high temperature curing cycle request needed by thermoset materials. The use of a new pad designed to be more environmental-friendly and the evaluation of root damage were the two most innovative points of our work which represent the second study of noxious effects induced by wear debris on higher soil plants providing useful information for the environmental risk assessment of these pollutants.

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Table 1
Bulk pad elemental composition of from traditional and innovative brake pads. Data are expressed as percentage of weight. "–" indicates that the element is below the limits.

Element	Mass [wt%]	
	Traditional pad	Cementitious pad
C	48,2082	37,6156
Mg	7,5874	0,5899
Zn	7,3697	0,0551
Al	7,2268	4,1649
Fe	4,9209	19,2565
Sn	4877	3,1382
Si	4,1861	1,4562
S	3,3543	4,8503
Cu	3,2498	13,7555
Ca	3243	4,9112
Cr	2,0925	1,5013
F	1,8654	0,0656
P	0,6042	0,0151
K	0,544	0,9748
Bi	0,4358	–
Ti	0,1033	0,0379
Mn	0,0672	0,1952
V	0,0182	–
Ni	0,0125	0,0205
Cl	0,0117	0,0078
Sr	0,0063	0,0921
Co	0,0062	–
Pb	0,0036	0,0078
Ga	0,0025	–
Rb	0,0023	–
Mo	0,0009	1,7581
Ba	–	5,4287
Na	–	0,0855
Sb	–	0,0162

2. Materials and methods

2.1. Wear debris

Wear debris for each sample were generated from traditional (TBP) and cementitious (CBP) pads during a dedicated bench test.

A full-scale dynamometer adapted to measure wear particle emission was used to generate and collect wear particles [Perricone et al., 2015; Matějka et al., 2016]. The test procedure is modified SAE J2707 Method B based on six blocks of brake snubs, simulating actual urban driving conditions [Matějka et al., 2016].

A pair of phenolic low-metallic brake pads and a pair of cementitious based brake pads were used during the experiments with cast iron brake disc.

X-ray fluorescence spectroscopy (XRFS) was used to analyze the chemical composition of the brake linings of both pads (Table 1).

Debris were collected at the end of the dynamometer wear test procedure directly on a stainless tray placed below the dynamometer in the bench. Bigger debris were removed before the BPWD were transferred into glass Petri dishes for storage. BPWD were stored at 4 °C protected from light until needed.

2.2. Characterization of wear debris

Wear debris from each brake pad during the bench test was characterized for chemical composition, shape and dimension.

Chemical composition was obtained from SEM/EDXS (Zeiss EVO MA10 – INCA x-act) analysis on wear debris. Suitable amounts of materials were deposited directly on carbon tape and ten separate areas were measured for each sample. Chemical composition result was then averaged to obtain the final values.

Debris dry samples were then sputter-coated with a thin overlayer of gold prior to the inspection with Field Emission Scanning Electron Microscopy (FE-SEM, Zeiss ULTRA Plus, Carl Zeiss SMT) for the morphological analysis of debris. Gold layering is necessary to prevent sample-charging effects. SEM images were taken at an accelerated voltage of 5 kV.

The particle size distribution of debris was retrieved by Single Particle Extinction and Scattering (SPES) optical technology (CLASSIZER ONE v0.7, EOS S.r.l.). SPES was applied on each BPWD water suspension to identify and analyze separately the particles with different optical properties, as single particles with different refractive indices or aggregates [Potenza et al., 2016a; Potenza et al., 2016b; Potenza et al., 2017; Sanvito et al., 2017; Mariani et al., 2017].

2.3. Effects of wear debris on plant germination and root growth

The acute phytotoxicity test was done following the protocol of Baderna et al. [2014, 2015a] using cress (*Lepidium sativum*) seeds grown in artificial soil with increasing concentrations of wear debris obtained from cementitious or traditional brake pads.

Wear debris was added directly on OECD standard soil (5% peat, 10% kaolin and 85% sand) to concentrations of 0.1, 1, 5 and 10 g of debris per kilogram of soil and mixed with a plastic spatula to homogenize the matrix. Ten grams of BPWD-contaminated soil were plated in a Petri dish (100 mm). Demineralized water was added as hydrating agent. Samples were covered with a wet paper filter (Whatman®qualitative filter paper, Grade 1). Uniform distribution of debris was checked visually before and after water addiction to ensure that particles were not clustered. Ten seeds (MicroBioTests Inc., Belgium) were placed on the filter paper. Plates were then closed with a lid, sealed and incubated in the dark at 25 °C for 72 h. Each condition were replicated five times. Control plates were set up with soil hydrated with deionized water while boric acid (250 mg/kg) was used as positive control as previously reported [Martignon, 2009; Baudo, 2013].

After incubation, we recorded for each plate the numbers of germinated seeds and the root length. These data were then combined to derive the germination index (GI), as follows:

$$\mathrm{GI} = \text{germinated seeds} \times \text{root elongation}$$

For each test condition, the mean GI and the percentage GI (GI%) were calculated:

$$\%\mathrm{GI} = 100 \times (<\mathrm{GI\ PBWD\ contaminated\ soil} > / < \mathrm{GI\ OECD\ soil}>)$$

2.4. Effects of wear debris on root damage

Root damages were evaluated with Evans Blue (EB) staining following a modified method of Barker and Mock (1994). Evans Blue stains injured cells due to loss of plasma membrane integrity [Yamamoto et al., 2001; Tamas et al., 2006; Motoda et al., 2010].

For each treatment, the apical regions (1 cm) of ten cress roots were cut using a disposable cutting blade and gently washed in 0.1 mM $CaCl_2$ for 10 min prior to staining with 0.025% w/v Evans blue in 0.1 mM $CaCl_2$ for 30 min at 25 °C in the dark. After staining, roots were washed three times with 0.1 mM $CaCl_2$, then submerged in SDS 1% w/v (bleaching solution). Roots were left under stirring on the roll bar for 15 min, protected from light then homogenized for 30 s in the bleaching solution with a microhomogenizer. Stirring and homogenizing were done at room temperature. The suspension was centrifuged (1000 rcf, 10 min) to remove plant debris and the absorbance of Evans blue released was measured spectrophotometrically at 620 nm. Each treatment was done in triplicate. Roots exposed to hydrochloric acid (37% w/v for 2 h) were used as a positive control. Results were expressed as the fold change in EB release compared to the untreated control group:

$$\text{EB release} = \text{Abs treatment}/\text{Abs control}.$$

Table 2
Elemental composition of debris from traditional and innovative brake pads. Data are expressed as percentage of dry weight. "–" indicates that the element is below the limits.

Element	Debris from traditional pad (% dry weight)	Debris from cementitious pad (% dry weight)
C	10.4	4.09
O	17.03	21.21
Mg	1.30	0.42
Al	1.12	1.34
Si	1.53	1.50
P	0.14	–
S	0.80	1.69
K	0.13	0.57
Ca	0.73	1.56
Cr	0.68	0.68
Mn	0.37	0.22
Fe	58.04	56.30
Cu	3.01	5.83
Zn	2.80	0.35
Sn	1.82	1.68
Ni	0.01	0.03
Ti	0.09	–
Ba	–	1.98
Mo	–	0.55

2.5. Data analysis

Prism7 (GraphPad Software, Inc.) was used to perform all statistical analyses. Non-linear regression fits were used to obtain concentration-response curves. Two-way ANOVA coupled to Dunnett's or Sidak's post hoc tests was applied for statistical comparisons, setting significance as $p < 0.05$.

3. Results

3.1. Characterization of wear debris

The analyses showed a different elemental composition of debris from the two pads (Table 2). Debris from traditional brake pads had a higher of carbon (10.4 vs 4.09%), magnesium (1.03 vs 0.42%), manganese (0.37 vs 0.22%), iron (58.04 vs 56.30%) and zinc (2.80 vs 0.35%) contents, while the contents of oxygen (21.21 vs 17.03%), aluminum (1.34 vs 1.12%), sulfur (1.69 vs 0.8%), potassium (0.57 vs 0.13%), calcium (1.56 vs 0.73%), copper (5.83 vs 3.01%) and nickel (0.03 vs 0.01%) were higher in the cementitious brake pads. Phosphorus and titanium were found only in the debris from traditional pads while barium and molybdenum were exclusively present in cementitious pad debris.

No significant difference in terms of appearance and morphology was observed from SEM micrographs of BPWD from traditional (Fig. 1A and B) and cementitious (Fig. 1C and D) brake pads. Actually, for both materials, highly heterogeneous populations of particle material in size (from 1 to 10 μm), with shape like small plates, aggregated or not, were visualized from the traditional the 2× magnifications. Thus, it can be said that similar materials were obtained from traditional and cementitious processes and the in size and shape of the material were not expected to affect their corresponding toxicity.

The real Re S(0) and imaginary Im S(0) components of the forward scattered field, two independent particle optical properties, were provided for each single particle by SPES optical method [Potenza et al., 2016a; Potenza et al., 2016b; Potenza et al., 2017; Sanvito et al., 2017; Mariani et al., 2017]. Raw data could be represented as a two-dimensional histogram with log-log axes (Potenza et al., 2016a). Particles with different optical properties, e.g. size, refractive index, structure, aggregation state or shape, populate different region of the 2D histogram (Potenza et al., 2016b; Potenza et al., 2017; Sanvito et al., 2017). It is possible to classify and retrieve separately information on the different species of particles measured in liquids or in aerosols. Here, SPES has been exploited to obtain information on the size of the BPWD (Fig. 2). No significant difference resulted, confirming what previously seen with TEM analysis. Debris from both pads were comparable in dimensions and size distribution, particle number mean diameter (NMD) and volume mean diameter (VMD) of debris are reported in (Table 3.)

3.2. Effect of BPWD on plant growth and root damages

The impact of wear debris on plant growth was examined using seed of *Lepidium sativum* and considering germination rate, root elongation and root damages.

No significant differences were found in the germination rate of cress seeds with any treatment (Fig. 3A). Germination rate was 95 ± 5.8% in the untreated group and from 90 ± 10% to 100 ± 0% in seeds grown in soil contaminated with debris from both brake pads.

Root elongation was affected significantly by wear debris from traditional pads: seedlings were generally shorter in soil contaminated with those debris but root elongations were statistically different from untread groups only in plants exposed to the highest concentration (10 g/kg) of debris (root growth was 57.65 ± 15.84% of the control) (Fig. 3B).

The overall effect of wear debris on seedlings was also evaluated also by the derivation of the GI, representative of the overall influence on both germination and growth (Fig. 3C). There was a clear dose-response effect in seeds exposed to debris from traditional brake pads with an appreciable inhibition (> 40%) at the highest concentration ($p < 0.0001$) (Fig. 3D). Inhibition founds in these seeds can be classified as moderate toxicity according to the different classification criteria found in literature [Martignon, 2009; Kapustuka et al., 2006; Baderna et al., 2015b]. No effects were recorded in seedlings grown in soil contaminated with increasing concentrations of debris from the cementitious brake pads. Moreover, considering the effects measured at the same concentration of both treatments, length was significantly different in roots grown in soil with 5 and 10 g/kg ($p < 0.01$ and $p < 0.0001$ respectively).

Furthermore, in the seedlings grown in soils contaminated with debris from the traditional pads, numerous lateral radicles was found, while no additional radicles was seen in the control plants and in those exposed to debris from the cementitious pads (Fig. 4).

Evans Blue assay was done on the exposed seedlings to evaluate root damages as loss of plasma membrane integrity (Fig. 5). A concentration-response trend was found in cress seeds grown in soil with increasing concentrations of wear debris from traditional brake pad but only in the roots of seedlings exposed to 5 (fold change of 1.16 ± 0.09, $p < 0.001$) and 10 g/kg (fold change of 1.58 ± 0.07, $p < 0.0001$) the level of damages was statistically different from the untreated roots. On the other hand, root damages were found only in cress seeds grown in soil contaminated with 10 g/kg of wear debris from cementitious brake pads (fold change of 1.25 ± 0.07, $p < 0.001$).

Focusing on the comparison between the two debris, statistically relevant differences were found in the groups grown in soil with 5 ($p < 0.0001$) and 10 g/kg ($p < 0.0001$), suggesting that wear debris from traditional brake pads are more harmful than debris from cementitious brake pads.

Table 4 shows the amounts of six metals in soils with the addition of 5 and 10 g of wear debris per kg of on the basis of the chemical characterization. Copper and tin concentrations resulting from soil contamination with 5 and 10 g of debris from both types of pad exceeded the respective ecological soil screening level (Eco-SSL) and the Italian legal threshold for residential soil. Zinc level exceeded the reference value only when the debris from the traditional pad was added to the soils at the concentration of 10 g per kilogram.

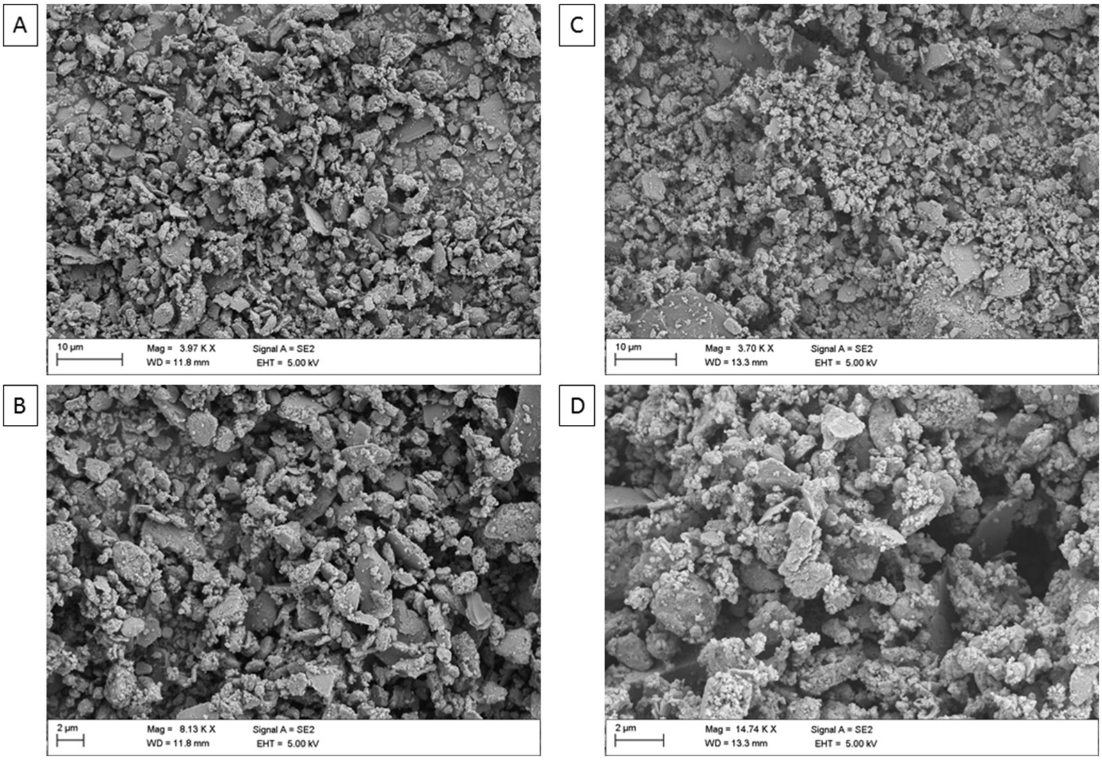

Fig. 1. Scanning Electron Microscopy of BPWDs at two magnifications with scale bars 10 μm (top pictures) and 2 μm (bottom pictures). Debris from a traditional brake pad (A-B) and from an innovative brake pad (C-D).

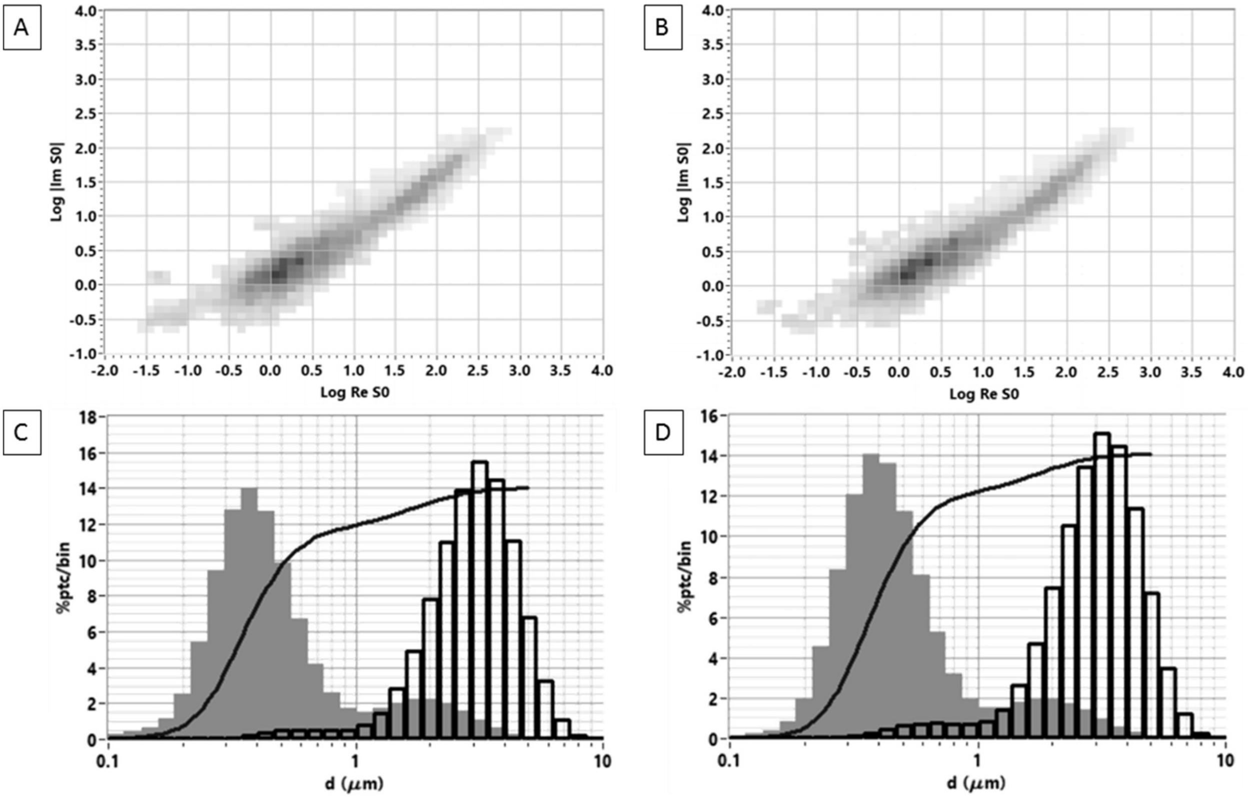

Fig. 2. BPWD characterization using the SPES analyzer. A-B: Traditional (A) and cementitious (B) brake pads. C-D Particle size distribution of BPWD from traditional (C) and cementitious (D) brake pads. The solid bars indicate the numerical size distribution and the empty bars illustrate refer to the volume particle size distribution.

Table 3
Particle Number (NMD), Volume mean diameters (VMD) and CV of BPWD from traditional and cementitious brake pads.

Brake pads	NMD [µm]	CV	VMD [µm]
Traditional BPs	0.56	1.16	2.90
Cementitious BPs	0.57	1	2.95

4. Discussion

Previous studies have estimated that particulate matter from traffic brakes are the most representative fraction in total dust collected from different road sites [Sanders et al., 2003; Hagino et al., 2016; Plachà et al., 2016; Plachà et al., 2017]. It has been reported that brakes can contribute between 11 and 21% to total traffic-related PM10 emissions in urban environments and half of the total brake wear may deposit on the road or in the roadside sites [Grigoratos and Martini, 2015; Janhäll, 2015]. Growing concern has arisen about the ecotoxicological potential of brake pad wear debris as these waste materials can be considered non-point sources of pollutants such as metals and various organic substances [Thorpe and Harrison, 2008; Dodd et al., 2014; Plachà et al., 2017]. These contaminants include both the structural components of the pads and compounds generated from friction and heat during braking [Sanders et al., 2003; Roubicek et al., 2008; Kukutschova et al., 2009; Kukutschova et al., 2010; Hagino et al., 2016; Plachà et al., 2017]. Once released into the environment, wear debris can interact with soil and water ecosystems after atmospheric transport and deposition. Few studies have been published considering the effects of traffic-related non-exhaust emission and only three focused on BPWDs highlighting possible adverse effects on bacteria, aquatic macrophytes and terrestrial plants [Kukutschova et al., 2009; Shupert et al., 2013; Dodd et al., 2014].

We investigated the influence of BPWD from traditional phenolic resin-based pads and innovative cement-based pads on cress seed germination, root growth and damage. Our experimental design reflects a potential environmental scenario in which the heaviest coarse debris are deposited near the emission point without being transported far from the source as it happens for the smaller and lighter particles (Chang et al., 2009; Ciudin et al., 2014). Cress (*L. sativum*), a dicotyledons plant, is often used as a model organism of fruits and vegetables [Martignon, 2009]. A previous study, also conducted by our group, indicated that cress is a very sensitive plant, particularly to heavy metals [Vidic et al., 2009; Nagajyoti et al., 2010; Visioli et al., 2014; Baderna et al., 2015b].

Seed germination and root elongation are investigated worldwide to assess phytotoxicity as they are key events for plant growth and interact with their environment and pollutants [Pignatti et al., 2001; USEPA, 2012; Dodd et al., 2014]. To define the overall impact of wear debris on seeds, we selected the combined germination index as previously done by our group [Baderna et al., 2014; Baderna et al., 2015a; Baderna et al., 2015b].

To our knowledge, the study by Dodd et al. [2014] is the only one focusing on debris effects on higher plants. It is important to note that our experimental conditions were different than from used in the Dodd study in which seedlings were in direct contact with the BPWDs or on a paper filter soaked with debris water extract. Our conditions are probably closer to the real situation, since the filter and the exposure to debris mixed in the soil may mitigate the toxic effect of the particles. Responses to the presence of obstacles such as the slowing of root growth or alterations to the development and orientation of the roots reported by Dodd et al., are not expected to occur in our experimental conditions.

Our results showed that higher concentrations of BPWD from

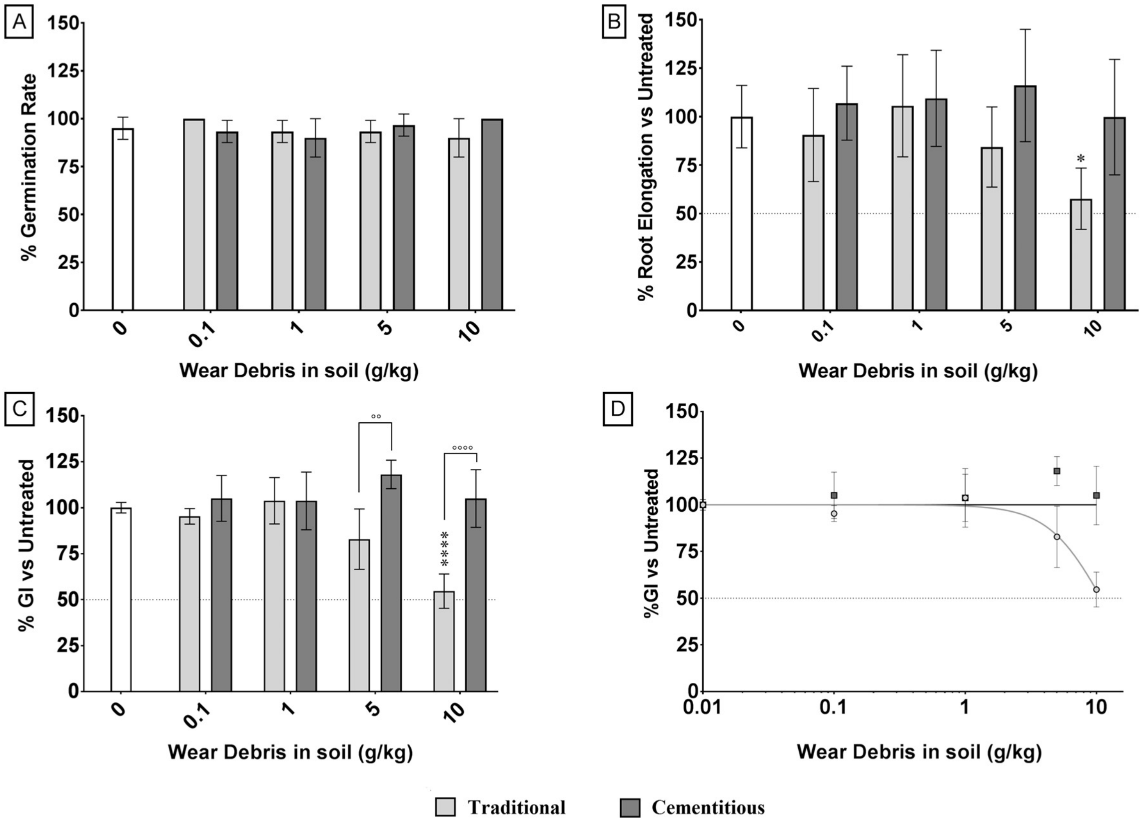

Fig. 3. Effects of wear debris on *Lepidium sativum*: A) Germination rate, B) Percentage root elongation, C) Germination index (%GI), D) Dose-response curve of %GI in cress seedling exposed to wear debris from traditional or innovative brake pads. 2-way ANOVA + Dunnett's post hoc: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ or 2-way ANOVA + Sidak's post hoc: °° $p < 0.01$, °°°° $p < 0.0001$.

Fig. 4. Effects of wear debris on *Lepidum sativum*: A) plant grown in untreated control OECD soil, B) seedling with lateral radicles from soil with debris from traditional brake pads.

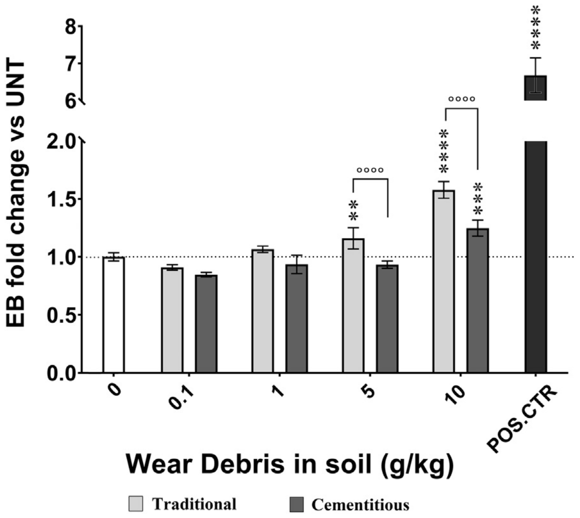

Fig. 5. Root damage in cress seedlings exposed to soil contaminated with wear debris: 2-way ANOVA + Dunnett's post hoc: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ or 2-way ANOVA + Sidak's post-hoc: °° $p < 0.01$, °°°° $p < 0.0001$.

traditional brake pads markedly lowered the %GI while no effects were found in seedlings exposed to cementitious-based debris. The lower %GI may be due to a decrease in the germination rate, to inhibition of the root elongation or to a combination of both effects.

The germination rate of cress seeds was not altered by increasing concentration of BPWD in soil, while root elongations were inhibited only in seedlings exposed to the highest concentration of debris from traditional brake pads. Comparable effects were reported by Dodd et al. [2014] on lettuce, wheat and soybeans finding that the debris particles from low-metallic brake pads had appreciable effects on root elongation but not on seed germination.

Lateral radicles were found in seedlings exposed to soil contaminated with debris from traditional brake pads, but not in control plants or in cress grown with debris from the innovative pads. Similar effects were found in soybean and lettuce exposed to water extract from low-metallic brake pads (Dodd et al., 2014). Dodd and coauthors suggested that the secondary lateral roots might be an early sign of toxicity due to copper as in the study by Nagajyoti et al. [2010], or morphological adaptations to increase the plant's surface and favor the absorption of iron as micronutrient [Hindt and Guerinot, 2012].

Several studies of about the molecular mechanisms underlying metal toxicity on plants have shown that these compounds can alter cell viability in root tips, causing loss of plasma membrane integrity and consequent inhibition of root growth [Yamamoto et al., 2001; Tamas et al., 2006; Motoda et al., 2010; Tistama et al., 2012]. We evaluated plasma membrane integrity in root tips with the Evans blue assay: wear debris from traditional pads damaged the roots of cress seedling's grown in soil contaminated with 5 and 10 g/kg debris, while significant alterations were found only in seeds exposed to 10 g/kg of debris from cementitious.

Because there were not significant morphological differences between both debris and hence toxicity was not related with their difference in shape or size, the overall phytotoxic effect can be attributed to the presence of heavy metals, some with non-negligible concentrations such as iron, copper and zinc. We also examine the metal levels resulting from soil contaminated with 5 or 10 g/kg of debris. We selected six metals with reference values from among the U.S. EPA Eco-SSL or within the legal limits set by the Italian Consolidated Environment Act. Copper, zinc and tin were higher than the reference values, in particular higher than the Eco-SSL which can be considered risk-based ecological soil screening levels for plants [USEPA, 2005]. It is important to underline that the hypothetical BPWD deposition might only be reached after prolonged time of deposition [0.6–1 mg total dry deposited particulate matter / (m^2 day) in winter, unpublished data] only considering the accumulation of particles in soil. We can hypothesize that debris from both pads at environmental levels could not be able to induce adverse effects on germination, root elongation and root integrity following acute or short-term exposures as evidenced by

Table 4

Heavy metals in soil contaminated with debris and comparison with their reference values. a) Italian Consolidated Environment Act (D.lgs152/2006), b) USEPA, 2007a, 2007c) USEPA, 2007b, 2007d) USEPA, 2007c; e)USEPA, 2007d.

Traditional pads				Cementitious pads			Reference values	
Element	% BPWD dry weight	Concentration in soil contaminated with 5 g/kg of BPWDs (mg/kg)	Concentration in soil contaminated with 10 g/kg of BPWDs (mg/kg)	% BPWD dry weight	Concentration in soil contaminated with 5 g/kg of BPWDs (mg/kg)	Concentration in soil contaminated with 10 g/kg of BPWDs (mg/kg)	Eco-SSL (mg/kg)	Italian soil threshold concentration (mg/kg) [(a)]
Cr	0.68	34	68	0.68	34	68	–	150
Mn	0.37	18.5	37	0.22	11	22	220 [(b)]	–
Cu	3.01	**150.5**	**301**	5.83	**291.5**	**583**	70 [(c)]	120
Zn	2.8	140	**280**	0.35	17.5	35	160 [(d)]	150
Sn	1.82	**91**	**182**	1.68	**84**	**168**	–	1
Ni	0.01	0.5	1	0.03	1.5	3	38 [(e)]	120

Bold indicates values higher than reference values.

the results we obtained in the experiments at the lower concentrations.

In conclusion, an acute phytotoxicity test with cress seeds can be used to assess the toxicity of brake pad wear debris. BPWDs from simulated urban driving conditions affect growth and damage cress seedlings, particularly when the plants grow in soil contaminated with high doses of debris. The results also suggest that debris from the traditional phenolic resin-based pads tested are more toxic than one from innovative cementitious pads, even if they are comparable in shape and size. Further studies are required to clarify the potential risk associated with the release of wear debris into the environment, in particular the possible chronic effects and the noxious effects on other soil organisms including soil microbial community whose sensitivity to metal pollution has already been demonstrated (Liao and Xie, 2007; Wang et al., 2007; Nwuche and Ugoji, 2008; Touceda-González et al., 2017; Zhang et al., 2017; Frossard et al., 2018).

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