Evaluation of toxicity and genotoxicity of concrete cast with steel slags using higher terrestrial plants

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Abstract

Steel slags (SS), by-products of the steel industry, may be used as recycled materials. However, their reuse may generate the potential release of harmful compounds into the environment. The aim of this study was to assess the potential impact of concrete mixtures cast with SS as partial replacement of natural aggregate on the terrestrial ecosystem, in terms of toxicity and genotoxicity, by using a battery of bioassays composed of higher plants. Four concrete mixtures, each one cast with the replacement of natural aggregates by 30% of four different SS and a mixture with natural aggregates only (reference concrete) were submitted to the monolithic leaching test (UNI EN 15863). The leachates were assayed for phytotoxicity by using seeds of *Lepidium sativum*, *Cucumis sativus*, and *Allium cepa*. The emerged seedlings of *L. sativum* and *A. cepa* were used for the evaluation of the DNA damage through the Comet test. The genotoxicity of the leachates was also analysed by means of bulbs of *A. cepa* applied through the Comet and the chromosomal aberrations tests. None of the samples caused phytotoxic effects towards the tested seeds. Rather, almost all the samples support the seedlings emergence, demonstrated by germination indexes (GI%) between 90% and 120%. Moreover, two leachates from concrete cast with SS and the one from the reference concrete were able to promote the germination and root elongation of *C. sativus* and *A. cepa*. DNA damage in *L. sativum* and *A. cepa* seedlings was significantly increased only by the sample from reference concrete, while the four leachates from concrete cast with SS did not differ by the controls. Conversely, the DNA damage on *A. cepa* bulbs was significantly improved by leachate from reference concrete, but also by that from a sample of concrete with SS. Moreover, all the leachates caused the rise of chromosomal aberrations in bulbs of *A. cepa*. Despite some genotoxic effects of concrete on plant cells, the partial replacement of SS does not seem to make concrete any more hazardous than the reference one in terms of global toxicological assessment, suggesting the potential use of SS as reliable recycled material, leading to a reduction of the impact of the anthropic activities on the environment.

1 Introduction

Steel slags (SS) are significant by-products of the steel industry. The European production in 2018 amounted to 16.3 million tonnes (Harder 2020). SS are used as recycled materials in many applications such as road base, asphalt mixtures, and in the construction industry, allowing to reduce the final disposal in landfills (Euroslag 2019; Collivignarelli et al. 2020). However, the reuse of this material may generate potential release of harmful compounds for the environment and humans (Primavera et al. 2016).

Few studies analysed the SS toxicity due to their recovery/disposal. To address this concern, which is not yet clearly defined from the regulatory point of view, previous studies were developed on the impact of granular SS and natural aggregates leachates on living organisms (bacteria, animals, plants), using an integrated chemical-biological approach (Benassi et al. 2019; Alias et al. 2021). The investigation of the impacts of concrete cast with a partial replacement of natural aggregates by SS represents a step forward. The direct contact of concrete products with the ground poses many ecotoxicological and
toxicological concerns. The cementitious materials are known to be able to release toxic substances (heavy metals, organic compounds, etc.) in the aquatic compartment (Hillier et al. 1999; Andersson 2002). Moreover, building materials are often treated with biocides against microbial colonization and biodeterioration (Reiß et al. 2021).

Soil is a complex matrix, with strong interactions with the other environmental matrices (air and water) and a close link to the anthroposphere (Norra 2009). In particular, urban soils are the first interface of several detrimental compounds contamination (Modabberi et al. 2018).

A broad range of biological models are available to study the contamination of the terrestrial compartment. Toxicity assays are based on soil microorganisms (among which *Arthrobacter globiformis*), annelids such as earthworms (*Eisenia fetida/Eisenia andrei*), arthropods as springtails (*Folsomia candida*), and plants, such as barley (*Hordeum vulgare*), cress (*Lepidium sativum*), and onion (*Allium cepa*). Many others monocotyledons (*Segale cereale, Loliurn perenne, Oryza sativa, Avena sativa, Triticum aestivum, Sorghum bicolor, Zea mays*) and dicotyledons (*Sinapis alba, Brassica napus, Raphanus sativus, Brassica rapa spp., Brassica campestris, Trifolium orinithopodioides, Lactuca sativa, Lycopersicon esculentum, Phaseolus aureus*) are also proposed as model organisms for toxicity studies (APAT 2004). Genotoxicity assays are mainly based on the same plants, including *Allium cepa, Arabidopsis thaliana, Glycine max, Hordeum vulgaris, Tradescantia spp., Vicia faba*, and *Zea mays* (Ma et al. 2005; de Souza et al. 2016).

The application of toxicity and genotoxicity short-term assays together with the chemical analysis has enhanced importance in the characterization of a wide range of materials, such as nanomaterials (Shvedova et al. 2010; De Marchi et al. 2018; Kisin et al. 2020), industrial adhesives (Cavallo et al. 2022), and construction additives (Baderna et al. 2015; Heisterkamp et al. 2019).

The variousness of endpoints and/or of organisms is a trait of the toxicological investigation. Tests are routinely combined to reduce uncertainty (Bierkens et al. 1998). Different organisms belonging to almost three trophic levels are required for ecotoxicity and genotoxicity evaluations of several environmental matrices, such as waste (Pandard et al. 2006; Alias et al. 2021), contaminated soils (Bierkens et al. 1998; Fernández et al. 2010), drinking water (Ceretti et al. 2016; Alias et al. 2022b).

Plant-based assays are part of these analytical batteries, often included because of the good correlations with other plants and animals systems, including humans (Tedesco and Laughinghouse IV 2012; Reis et al. 2017). For these reasons, the results of such batteries have been combined for the construction of risk matrices (Marmiroli et al. 2022) and environmental footprint maps (Pedrazzani et al. 2020; Bertanza et al. 2021).

Terrestrial plants represent the largest interface between the environment and the biosphere, and the plant-based assays have several advantages: great sensitivity, ecological pertinence, ease of execution and low-cost. Therefore, plants are useful tools to thoroughly investigate the environmental degradation caused by the most conventional soil pollutants, like pesticides (Rose et al. 2022; Menzyanova et al.
2022), urban and industrial wastewater (Bertanza et al. 2021; Chowdhary et al. 2022; Singh et al. 2022), as well as the emerging ones, including plastic particles (Bouaicha et al. 2022; Wang et al. 2022), carbon quantum dots (Vijeata et al. 2022), and rare earth elements (Egler et al. 2022). Furthermore, the combined effect of different pollutants (bisphenol A and titanium dioxide nanoparticles) on agricultural environment have been investigated from the toxicological to the proteomic level (Huang et al. 2022). Plant-based assays could also be applied to the quality assessment of both drinking water (Feretti et al. 2012), and air (Ceretti et al. 2015; Feretti et al. 2019; Vieira et al. 2022).

Among others, the *Allium cepa* is an excellent model *in vivo*, suitable for both the toxicity and genotoxicity biomonitoring (Tedesco and Laughinghouse IV 2012), easily available in two forms, seeds and bulbs. The dimension and number of *A. cepa* chromosomes (2n = 16) enable a (relative) simple analysis of microscopic parameters such as mitotic index, chromosomal aberrations (including c-mitosis, bridges, fragments, buds), and micronuclei (Ragazzo et al. 2017; Felisbino et al. 2018; Camilo-Cotrim et al. 2022; Gupta et al. 2022). Furthermore, *A. cepa* is the most used higher plant for the investigation of early damage through the single-cell gel electrophoresis (or Comet) test (Lanier et al. 2015). Because of the abovementioned characteristics, *A. cepa* is largely applied to determine the cytotoxicity and genotoxicity of soil contaminants such as pesticides (Rosculete et al. 2019; Camilo-Cotrim et al. 2022), veterinary drugs (de Souza et al. 2022), biosorbents (Pantano et al. 2021), as well as phytotoxicity and genotoxicity of different sludges (Santos et al. 2022).

An innovative toxicological workflow has been recently reported by some authors who investigated the toxicity and the genotoxicity of chemicals on plants by using the same organisms subsequently submitted to different evaluations. Passatore and colleagues studied the effects of bismuth exposure on *Lepidium sativum* seedlings (Passatore et al. 2022). Similarly, the physiological and genotoxic effects of phthalate on *Lemna minor* and *Spirodela polyrhiza* were investigated by Pietrini and collaborators (Pietrini et al. 2022).

In this research a similar synergistic procedure using toxicity and genotoxicity plant-based assays is proposed to assess the environmental impact of reference concrete mixtures cast with natural aggregates and concrete cast with SS as partial substitute of natural aggregate.

### 2 Material And Methods

#### 2.1 Materials

Four samples of electric arc furnace steel slags (SS) were collected from four northern Italian steel-making plants. Samples were coded as: A, B, C, and D. Particle size ranged from 1 to 5 cm, whereas the density ranged between 2.5 and 3.3 g/cm³. SS were stored from the factories in open, unprotected areas, subjected to a maturation process for at least 3 months after production, leading to the natural carbonation of alkaline residue. A sample of natural aggregates (NA) was collected from a quarry of Northern Italy.
Four concrete mixtures, called steel slag concrete, were cast with the use of Portland cement (13% w/w) and a partial substitution (30%) of natural aggregate with SS (CSS); moreover, a reference concrete mixture was cast with natural aggregates only (CNA) and the same amount of Portland cement. The cement/water ratio was 0.48 and the concrete density was about 2470 kg/m$^3$. The casts were naturally dried until constant weight before the analysis (around 30 days).

### 2.2 Leaching test

Leaching tests were performed on CSS and CNA according to the standard procedure on monolithic waste (UNI EN 15863 2015), only for the first two extraction steps for a total duration of 24 h, as reported in UNI 10802 (UNI 10802 2013). Tests were performed on soaked concrete blocks in demineralized water (leachant) at a liquid to surface area ratio of 8 mL/cm$^2$. The leachant was renewed after 6 and 18 h of contact (CSS-6h, CSS-18h, and CNA-6h, CNA-18h). Leachates were filtered (membrane pore size 0.45 µm) and stored at 4°C.

### 2.3 Plant-based assays

The toxicity and the genotoxicity of leachates were assayed by using several plants.

#### 2.3.1 *Lepidium sativum, Cucumis sativus, and Allium cepa* seed germination and root elongation tests

The assays were performed according to the Italian Environmental Agency guidelines (APAT 2004). Briefly, seeds of *Lepidium sativum, Cucumis sativus*, and *Allium cepa* not treated with fungicides, were preliminary checked for vitality in distilled water in the dark at 25 ± 1°C (germination rates > 90%). Leachate solutions were tested without any dilution, and the distilled water was used as negative control. Three replicates per treatment were arranged by wetting with a Whatman no. 1 filter paper with 2 mL of each solution. Ten seeds for each replicate were distributed on the filter. The three dishes of each replicate were packed into a tightly closed plastic bag and incubated at 25 ± 1°C in the dark for 72 h. At the end of the incubation time, complete sprouts (≥ 1 mm) and root lengths were evaluated. Results were expressed as mean germination index (GI) ± standard error (SE).

#### 2.3.2 Lepidium sativum and Allium cepa seedlings Comet tests

At the end of the germination and elongation test, thirty seedlings exposed to the 6-h fraction of each leachate were collected in an ice-cold dish. Negative and positive control were performed using distilled water and methyl methanesulfonate (MMS, 10 mg/L), respectively. The tips were finely chopped with a scalpel and 500 µL of ice-cold nuclei isolation buffer (200 mM Tris, 4 mM MgCl2.6H2O, 0.5% Triton-X) were added. The suspension was let to sediment on ice for few minutes. 180 µL of supernatant were diluted 1:1 in low melting agarose (LMA, 0.7%). The suspension was distributed on an agarose-coated glass slide and a coverslip was immediately placed on the top of the cell-gel mixture. The slides were then placed for 30 min at 4°C, to allow the solidification of the agarose. After that, the coverslips were
gently removed and the samples were subjected to 1 h unwinding (pH = 12.3) and 20 min electrophoresis (pH = 12.3, 0.8 V/cm, and 25 V at limit). The slides, stained with GelRed Nucleic Acid Gel Stain (Biotinum), were examined under a fluorescence microscope (Olympus CX 41RF) equipped with a BP 515–560 nm excitation filter and an LP 580 nm barrier filter. Levels of DNA damage were evaluated by the comet parameter “tail intensity” (percentage of DNA migrated in the tail) detected by an automatic image analysis software (Komet 5, Kinetic Imaging Ltd, UK). The statistical analysis was performed by using ANOVA univariate and Dunnett’s multiple comparison test, where p < 0.05 was considered significant.

### 2.3.3 *Allium cepa* bulbs toxicity test

The toxicity test was performed to determine the doses to be used in the genotoxicity test (Fiskesjö 1985; Rank et al. 2002). Equal-sized young onion bulbs were purchased from the local market without any treatment. Twelve bulbs were exposed for 72 h in the dark to different sample solutions (100, 50, 10, and 1%). Distilled water was used to dilute the samples and as a negative control. The roots mean length was used to calculate the EC50 value (Fiskesjö 1995) by Microsoft Excel (2019). Other toxicity parameters (turgidity, consistency, colour change and root tip shape) were also evaluated.

### 2.3.4 *Allium cepa* bulbs Comet test

Three equal-sized young onion bulbs per sample were submitted to a 48-h pre-germination period in Rank’s solution, then exposed for 24 h to undiluted samples based on the results obtained in the above-described toxicity test on bulbs. Negative and positive controls were conducted using Rank’s solution and methyl methanesulfonate (MMS, 10 mg/L), respectively. Fifty meristematic root tips (5 mm long) were cut and collected in an ice-cold dish. The procedure described above for seedlings was entirely followed for bulbs, too. Each experiment was conducted in duplicate. The statistical analysis was performed by using ANOVA univariate and Dunnett’s multiple comparison test, where p < 0.05 was considered significant.

### 2.3.5 *Allium cepa* bulbs chromosomal aberrations test

The *Allium cepa* test for the evaluation of the chromosomal aberrations (CA) (Rank 2003) was performed by using six onion bulbs per sample. After 48 h pre-germination in Rank’s solution, the bulbs were exposed for 24 h to undiluted samples based on the results obtained in the above-described toxicity test on bulbs. The roots were cut, fixed in 1:3 acetic acid-ethanol for 24 h and stored in 70% ethanol. Five roots of each sample were considered for the microscopic analysis after staining with 2% acetic orcein. 5,000 cells were scored for the mitotic index (MI), the measure of the cell division rate. Following recommendations, samples with MI lower than 1% were not included. 1,000 cells in mitosis were scored for the CA frequency. The different types of aberrations were classified in three main categories: fragments, rings, sticky chains, bridges as “direct DNA damage” (DDD), laggards, binucleated, polar slips, multipolar, c-mitosis as “mitotic spindle defects” (MSD), and buds as “genic amplification” (GA). Rank’s solution was used as a negative control. A positive control was performed using maleic hydrazide (10 mM). The experiments were performed in duplicate. The statistical analysis was performed by using χ² test, where p < 0.05 was considered significant.
3 Results

3.1 Phytotoxicity

The germination and the root elongation of seeds of three plant species (*Lepidium sativum*, *Cucumis sativus* and *Allium cepa*) were evaluated (Fig. 1). Both fractions of all the samples derived from the leaching tests (6h and 18h) exerted a non-toxic effect towards the plant species, demonstrated by the germination indexes around 100%, and comprised between 80% and 120% (Da Ros et al. 2018). Moreover, samples CSS-C-6h and CNA-6h biostimulated *C. sativus* (GI% = 121.3 ± 3.1 and 133.2 ± 8.9, respectively), as did sample CNA-18h on *A. cepa* (GI% = 124.3 ± 4.0).

3.2 DNA damage on *Lepidium sativum* and *Allium cepa*

The DNA damage was evaluated on seedlings of *Lepidium sativum* and *Allium cepa* which previously underwent the germination test and on bulbs of *A. cepa* by using the Comet test.

Due to the absence of differences between the two fractions of leachates (6 and 18 hours of leaching), the Comet tests were performed only on seedlings germinated on the 6-h fractions (Table 1). DNA damage in *L. sativum* seedlings was significantly increased only by the sample cast with natural aggregates (TI = 18.9 ± 0.2), while the four leachates of concrete cast with SS did not significantly differ by the control. The same trend was demonstrated in *A. cepa* seedlings, which were significantly damaged only by the leachate of concrete cast with natural aggregates (TI = 12.1 ± 1.6).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tail intensity ± SD</th>
<th>Lepidium sativum</th>
<th>Allium cepa</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSS-A-6h</td>
<td>8.7 ± 0.7</td>
<td>3.3 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>CSS-B-6h</td>
<td>6.3 ± 4.2</td>
<td>3.5 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>CSS-C-6h</td>
<td>9.9 ± 6.9</td>
<td>4.8 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>CSS-D-6h</td>
<td>10.2 ± 3.8</td>
<td>9.6 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>CNA-6h</td>
<td>18.9 ± 0.2 *</td>
<td>12.1 ± 1.6 *</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>5.3 ± 0.4</td>
<td>5.3 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>31.3 ± 10.8</td>
<td>25.4 ± 6.2</td>
<td></td>
</tr>
</tbody>
</table>

SD: standard deviation; Statistically significant versus negative control according to Dunnett’s test: *p < 0.05.
Due to the absence of toxicity in *A. cepa* bulbs, in terms of root elongation (Table 1S) the DNA damage was assessed on undiluted samples. The Comet test on *A. cepa* bulbs revealed a significant DNA damage in onions treated with leachates from CNA and from CSS-C (Table 2). The damage was similarly induced, for both samples, by the two leachates derived from the leaching tests (at 6h and 18h).

**Table 2**

Comet test of undiluted leachates of steel slags concrete (CSS) and reference concrete (CNA) in *Allium cepa* bulbs.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tail intensity ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSS-A-6h</td>
<td>6.0 ± 0.3</td>
</tr>
<tr>
<td>CSS-A-18h</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>CSS-B-6h</td>
<td>3.9 ± 0.6</td>
</tr>
<tr>
<td>CSS-B-18h</td>
<td>5.3 ± 3.8</td>
</tr>
<tr>
<td>CSS-C-6h</td>
<td>11.7 ± 0.4 *</td>
</tr>
<tr>
<td>CSS-C-18h</td>
<td>9.8 ± 1.9 *</td>
</tr>
<tr>
<td>CSS-D-6h</td>
<td>6.2 ± 0.8</td>
</tr>
<tr>
<td>CSS-D-18h</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>CNA-6h</td>
<td>7.2 ± 0.7 *</td>
</tr>
<tr>
<td>CNA-18h</td>
<td>7.1 ± 0.1 *</td>
</tr>
<tr>
<td>Negative control</td>
<td>3.5 ± 1.5</td>
</tr>
<tr>
<td>Positive control</td>
<td>16.4 ± 2.6</td>
</tr>
</tbody>
</table>

SD: standard deviation; Statistically significant versus negative control according to Dunnett’s test: *p* < 0.05

### 3.3 Chromosomal aberrations on *Allium cepa*

The genotoxicity was also evaluated in terms of chromosomal aberrations on *A. cepa* bulbs. The frequency of CA was significantly improved by all the leachates, without affecting the mitotic indexes (ranging from 10.2 to 12.2%) (Table 3). In particular, the leachates from CSS-B-6h and CSS-D-6h induced the greatest increase of total CA (25.8 ± 7.7, and 25.4 ± 6.6, respectively). Moreover, for both mixtures the 6h-fractions were able to induce significantly more CA than the respective 18h-fractions.
Table 3
Mitotic index (MI) and chromosomal aberrations (CA) frequency of undiluted leachates of steel slags concrete (CSS) and reference concrete (CNA) in *Allium cepa* bulbs.

<table>
<thead>
<tr>
<th>Sample</th>
<th>MI (%)</th>
<th>CA (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSS-A-6h</td>
<td>10.6</td>
<td>17.0 ± 3.8 *</td>
</tr>
<tr>
<td>CSS-A-18h</td>
<td>11.3</td>
<td>15.4 ± 1.3 *</td>
</tr>
<tr>
<td>CSS-B-6h</td>
<td>11.3</td>
<td>25.8 ± 7.7 *§</td>
</tr>
<tr>
<td>CSS-B-18h</td>
<td>10.8</td>
<td>20.2 ± 2.3 *</td>
</tr>
<tr>
<td>CSS-C-6h</td>
<td>10.2</td>
<td>19.0 ± 5.3 *</td>
</tr>
<tr>
<td>CSS-C-18h</td>
<td>11.1</td>
<td>21.2 ± 4.4 *</td>
</tr>
<tr>
<td>CSS-D-6h</td>
<td>10.2</td>
<td>25.4 ± 6.6 *§</td>
</tr>
<tr>
<td>CSS-D-18h</td>
<td>12.2</td>
<td>18.0 ± 4.8 *</td>
</tr>
<tr>
<td>CNA-6h</td>
<td>11.3</td>
<td>19.4 ± 4.4 *</td>
</tr>
<tr>
<td>CNA-18h</td>
<td>11.8</td>
<td>17.6 ± 6.0 *</td>
</tr>
<tr>
<td>Negative control</td>
<td>10.7</td>
<td>10.6 ± 1.9</td>
</tr>
<tr>
<td>Positive control</td>
<td>7.8</td>
<td>37.8 ± 7.9</td>
</tr>
</tbody>
</table>

Statistically significant versus negative control according to χ² test: *p < 0.05; Statistical difference between fractions according to χ² test: § p < 0.05

Three types of chromosomal aberrations were identified: direct DNA damage (DDD), mitotic spindle defects (MSD), and genic amplification (GA) (Fig. 2). Generally, GA mechanisms impacted least the total aberrations (from 11.8–25.7%). The DDD frequency was within 11.8% and 34.7%, with the only exception of cells treated with CSS-D where the direct DNA damage was greater than 40% (40.9% and 43.3%, respectively for both fractions of CSS-D). Finally, the predominant processes were the MSD, with significant contributions well above 50% (p < 0.05 according to χ² test) for the majority of the samples, with the few exceptions of CSS-B-18h, CSS-D-6h and CSS-D-18h.

4 Discussion

Leachates from concrete cast with a partial substitution of SS and with NA only were characterized for phytotoxicity and genotoxicity through a plant-based approach to evaluate their impact on terrestrial compartment.

The phytotoxicity test on *Lepidium sativum*, *Cucumis sativus* and *Allium cepa* seeds did not reveal any concerning aspect of the tested samples. The germination indexes were all around 100%, and generally comprised between 80% and 120%, which represent values not different from the negative control, as
reported by Da Ros and colleagues (Da Ros et al. 2018). The sensitivity of the used macrophytes was comparable, with the few exceptions of a slight effect of biostimulation induced by samples CSS-C-6h and CNA-6h in *C. sativus* and sample CNA-18h in *A. cepa*. Likewise, the evaluation of root elongation in *A. cepa* bulbs revealed the absence of toxicity. The genotoxicity was assessed through two main end-points: the DNA strands breaks and the chromosomal aberrations, which represent the early-stage, reparable damages, and the stable, inheritable defects, respectively. The DNA damage evaluated on the emerged seedlings of *L. sativum* and *A. cepa*, was significantly increased only by the leachate from the reference concrete (CNA). Interestingly, the response was comparable on both plant species. The same early damage appraised on root cells of bulbs of *A. cepa* was significantly raised by the leachates from CNA and CSS-C. Regarding the assessment of stable damages, all samples caused the significant increase of the frequency of chromosomal aberrations. Notably, the predominant mechanism-of-action was attributable to mitotic spindle defects, and only secondarily to direct DNA damages.

The different results in terms of DNA damage between seedlings and bulbs of *A. cepa* are likely due to the sensitivity of the two forms of the plant, which represent distinctive physiological status of the same organism. Seeds, defined as the mature, fertilized ovules of flowers that contain dormant embryos and food stores awaiting germination (Lopez and Barclay 2017), are protected from the external environment by several tegumentary barriers. Moreover, the emerging radicle, or primary root, is an immature structure, not yet completely able to accomplish its tasks. All these aspects contribute to making the seeds less susceptible than the bulbs to the external inputs. Indeed, the genotoxicological damages detected in seeds might be attributable to a mixture present in the leachate from reference concrete more active towards the genome and/or more able to interact with the seedling structures. The analysis of DNA damage on onion bulbs confirmed these data and additionally demonstrated the genotoxicity of a sample derived from concrete cast with a partial substitution of SS, by virtue of the bulbs’ greater susceptibility. For the same reason, the chromosomal aberrations detection in *A. cepa* well highlighted the genotoxic aptitude of all samples.

Plants, as primary producers, are the first and the most important step in the food chain of each ecosystem. The direct contact with the matrix, and the water-soluble substances uptake capability enables them to strongly interact with pollutants. For these reasons, plants represent useful toxicological models for environmental studies, especially on soil and water, where they could be applied *in situ*, *in vivo* and *in vitro* settings. Furthermore, different parts of plants (e.g. leaves, meristems, seeds, pollen) and several end-points could be used to study many toxicological traits (Grant 1994; Leme and Marin-Morales 2009; Iqbal 2016). It is thus evidenced how the integration between different biological tests gives the possibility of covering several end-points, allowing for a detailed comprehension of the effects on the higher plants and an evaluation of the environmental hazardousness of the tested materials. Moreover, the coherence between the results from a battery of plant assays supports the obtained data themselves, allowing for a more certain identification of the most concerning samples. Additionally, and even more importantly, the biological assays are independent from a pre-determined analytical schedule, which could not necessarily completely describe a sample. Indeed, we previously experienced that leachates from different granular SS and NA, even if chemically characterized by full compliance with the
legislation requirement, demonstrated toxic effects towards several biological systems (Benassi et al. 2019; Alias et al. 2021). Likewise, we studied leachates from concrete cast with NA and with a partial substitution of SS that, again, were in complete conformity with the reference limits, but could impact on living organisms (Alias et al. 2022a).

The presented methodology led to an evaluation of the impact of concrete samples on the higher plants to allow their potentially reuse for building applications. The utilization of SS in partial replacement of natural aggregate allows to obtain concrete with good workability and mechanical properties (i.e., compressive strength and modulus of elasticity) compatible with their use in civil constructions (Rondi et al. 2016; Diotti et al. 2021). Not least, the reuse of SS leads to a reduction of the impact of the anthropic activities on the environment, mainly through the preservation of natural resources and the reduction of the waste landfill.

5 Conclusions

These results demonstrated that despite some genotoxic effects of concrete on plant cells, the partial substitution of SS does not seem to make concrete any more hazardous than the reference one in terms of global assessment (toxicological, environmental, economic), thus suggesting the potential use of SS as reliable recycled material. Furthermore, this study confirmed that the plant-based approach is a useful tool for the toxicological characterization of recycled materials intended to have a prolonged contact with soil and water.

Declarations

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Ethical Approval

Not applicable

Consent to Participate

Not applicable

Consent to Publish

Not applicable

Authors Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Carlotta Alias, Ilaria Zerbini, Laura Benassi and Donatella Feretti. The first
draft of the manuscript was written by Carlotta Alias, Ilaria Zerbini and Donatella Feretti and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Competing Interests**

No potential conflict of interest was reported by the authors

**Availability of data and materials**

Not applicable

**References**


Germination Index (GI) expressed as percentage in *Lepidium sativum*, *Cucumis sativus* and *Allium cepa* treated with undiluted leachates of steel slags concrete (CSS) and reference concrete (CNA). Data are expressed as mean GI% ± SE.
Figure 2

Frequency distribution of the three categories of chromosomal aberrations: direct DNA damage (DDD), mitotic spindle defects (MSD), and genic amplification (GA).

Supplementary Files

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- Table1S.docx