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Evaluation of the Ecotoxicological Potential of Fly Ash and Recycled Concrete Aggregates Use in Concrete

Patrícia Rodrigues ¹, José D. Silvestre ^{1,*} , Inês Flores-Colen ¹ , Cristina A. Viegas ² ,
Hawreen H. Ahmed ^{1,3} , Rawaz Kurda ^{1,3}  and Jorge de Brito ¹

¹ CERIS, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal; patricia.rodrigues@tecnico.ulisboa.pt (P.R.); ines.flores.colen@tecnico.ulisboa.pt (I.F.-C.); hawreen.a@gmail.com (H.H.A.); rawaz.kurda@tecnico.ulisboa.pt (R.K.); jb@civil.ist.utl.pt (J.d.B.)

² Bioengineering Department and iBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisbon, Portugal; cristina.viegas@tecnico.ulisboa.pt

³ Department of Civil Engineering, Technical Engineering College, Erbil Polytechnic University, Kirkuk Road, Erbil 44001, Kurdistan Region, Iraq

* Correspondence: jose.silvestre@tecnico.ulisboa.pt; Tel.: +351-21-841-9709

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Abstract: This study applies a methodology to evaluate the ecotoxicological potential of raw materials and cement-based construction materials. In this study, natural aggregates and Portland cement were replaced with non-conventional recycled concrete aggregates (RA) and fly ash (FA), respectively, in the production of two concrete products alternative to conventional concrete (used as reference). The experimental program involved assessing both the chemical properties (non-metallic and metallic parameters) and ecotoxicity data (battery of tests with the luminescent bacterium *Vibrio fischeri*, the freshwater crustacean *Daphnia magna*, and the yeast *Saccharomyces cerevisiae*) of eluates obtained from leaching tests of RA, FA, and the three concrete mixes. Even though the results indicated that RA and FA have the ability to release some chemicals into the water and induce its alkalinisation, the respective eluate samples presented no or low levels of potential ecotoxicity. However, eluates from concrete mixes produced with a replacement ratio of Portland cement with 60% of FA and 100% of natural aggregates and produced with 60% of FA and 100% of RA were classified as clearly ecotoxic mainly towards *Daphnia magna* mobility. Therefore, raw materials with weak evidences of ecotoxicity could lead to the production of concrete products with high ecotoxicological potential. Overall, the results obtained highlight the importance of integrating data from the chemical and ecotoxicological characterization of materials' eluate samples aiming to assess the possible environmental risk of the construction materials, namely of incorporating non-conventional raw materials in concrete, and contributing to achieve construction sustainability.

Keywords: chemical characterization; concrete; construction material; ecotoxicology; fly ash; leachate; raw material; recycled aggregates; sustainability

1. Introduction

The interest in using building construction materials with better mechanical, durability, and environmental characteristics is continuously growing [1–6]. However, to obtain such properties, particularly in cement-based materials, some non-conventional raw materials (such as fly ash (FA) and recycled aggregates (RA)), with unknown hazard levels, are being incorporated. Therefore, it is important to determine the ecotoxicological potential of these innovative construction materials and of their raw materials, so that their use and handling are consciously made and contribute to the sustainability of buildings.

Toxicology is a multidisciplinary science that studies the harmful effects resulting from the interaction between a toxic agent and a biological system in order to predict the advent of these effects. This science encompasses several areas that, depending on the field, have different names, namely ecotoxicology (or environmental toxicology), which studies the toxic effects of xenobiotics on organisms living in ecosystems [7], and occupational toxicology (or human toxicology), which focuses on the study of the harmful effects caused by chemicals present in the workplace [8]. Toxicology can contribute to technological advancement through the implementation of safe conditions of exposure to given substances [9]. To improve construction sustainability, ecotoxicology may have an important contribution in the assessment of the environmental risk associated with the products to be used in the construction sector. For that purpose, it is necessary to evaluate the products' potential ecotoxicity using leaching tests, ecotoxicity tests, and chemical analyses. The assessment of the environmental contamination risk triggered by the contact between surface and groundwater and the materials is done through leaching tests, which allow assessing the mobility of hazardous substances in aqueous media and their environmental impact, although, by themselves, these tests do not reproduce a real situation. These tests should be supplemented with chemical analyses and ecotoxicity testing using biological parameters to understand the influence of the aqueous extracts (e.g., leachates, eluates) on living organisms.

Ecotoxicity tests using the bioluminescent marine bacterium *Vibrio fischeri* are often used because they are simple, fast, and reproducible. *Vibrio fischeri* cells emit light when they are healthy, and this luminescence can decrease when the bacterium cells are brought into contact with a toxic agent that causes inhibition of metabolism. This test can be used in the assessment of the potential ecotoxicity of, for instance, leachates or eluates from solid samples like construction materials or ashes [10]. The study by Tsiridis et al. [10] sought to analyse the toxicity of FA and of its elution products to determine the most sensitive bio test in the toxicological assessment and the most effective leaching process. These authors concluded that the *Vibrio fischeri* test (Microtox test) is suitable for the preliminary screening of solid waste eluate toxicity. However, comparison of data from tests performed with the former bacterium, a freshwater planktonic crustacean *Daphnia magna* and a freshwater planktonic rotifer *Brachionus calyciflorus* indicated *Daphnia magna* as the most sensitive test organism to assess the ecotoxicity of FA eluates [10]. The study also revealed that FA samples obtained from various coal-fired power plants contain heavy metals, namely: Cr, Cu, Mn, Ni, Pb and Zn. The results of the eluates' chemical analysis in accordance with EN 12457-2 [11] and with the toxicity characteristic leaching procedure (TCLP) [12] indicated that the chemical compounds present in FA can be transferred to the liquid phase depending on the pH of the leaching medium. Thus, the pH of the eluate was also considered an important factor in determining environment contamination and ecotoxicity of FA [10]. In general, the chemical analysis showed the elements Al, Ca, Fe, Mg, and Cr are the most frequently found in both solid samples and eluates of FA [10].

Choi et al. [13] proved *Daphnia magna* could be suitable to evaluate the ecotoxicity of leachate from concrete. The leachates of cement samples in acute toxicity test showed 100% *Daphnia magna* immobilization upon 24 h exposure. The authors pointed out the potential negative impact of alkali leaching from concrete into the aquatic environment [13].

The yeast species *Saccharomyces cerevisiae* is a model eukaryotic microorganism and has been used as a test organism in the assessment of the potential toxicity of pesticides and other environmental contaminants [14]. A study developed by Gil et al. [14] showed that this model is suitable for the development of simple low-cost toxicity tests, which can be useful to provide quick preliminary screening of environmental samples' potential toxicity level, before advancing further to ecotoxicity testing with more complex higher eukaryotes.

Kanare and West [15] studied the composition of leachates obtained from concrete mixes produced with two types of aggregates and four types of Portland cement. The authors used two leachants: acetic acid solution and deionised water. The results with acetic acid showed that Hg, Cr, Pb, and Ni were leached, whereas with the deionised water, only partial leaching of Hg, Cr, and Ni occurred.

Rankers and Hohberg [16] investigated various leaching tests on cementitious mortars with FA. The experiment included four types of tests: glass/polyethylene column test (pH 4 water with nitric acid); DEV-S4 batch test (demineralized water); bottle test (pH 4 water with nitric acid); and tank-leaching test (pH 4 water with nitric acid). The first two tests were considered not adequate to construction materials. In the third test, maximum toxic element concentrations were estimated and the results were considered reproducible. The diffusion leaching rates were calculated according to the fourth test, which was also considered reproducible.

Hillier et al. [17] investigated the long-term (up to 256 days) leaching of toxic metals into deionized water from cementitious mortars. The tested samples were analysed according to Directive 80/778/EEC [18] for different metals by using atomic absorption spectroscopy. Of the metals analysed, only vanadium leaching was found in poorly cured mixes [17].

Gwenzi and Mupatsi [19] studied the leachability of heavy metals from cement, sand, and coal ash into water. Total concentrations of Zn, Pb, and Mn in coal ash were lower or similar to those in the other materials, whereas total Fe and Cu were higher in coal ash. In general, the leached concentrations of Fe, Zn, Cu, Mn, and Pb from coal ash and from concrete made with it were found low and comparable to that of conventional concrete, suggesting leaching risk. The authors suggested that coal ash can be incorporated in concrete since it is not anticipated to have harmful impact on environmental and public health related to this constituent [19].

In conclusion, the literature review shows that the incorporation of alternatives (FA and RA) to replace conventional components (cement and natural aggregates (NA)) is insufficiently studied in terms of ecotoxicological potential [20]. Moreover, studies concerning concrete mixes with the simultaneous and high incorporation ratio of FA and RA to produce sustainable RA concrete are missing. In this context, this study focuses on the application of an innovative methodology developed in a previous study by the authors [21] that reveals the environmental profile and the potential ecotoxicological risk of raw materials and building materials. In the present study, the developed methodology was applied to three concrete mixes and to raw materials incorporated in them, namely RA and FA. This was intended to assess their environmental risk when considered unusable and sent to landfills. The ecotoxicity results obtained by applying the proposed methodology can be used independently or as part of life cycle assessment (LCA) studies. Generally, these risks are not considered in LCA studies, although being of high relevance to researchers developing innovative and sustainable materials.

2. Methodology

A global methodology was proposed in a previous study by the authors [21] for assessing the ecotoxicological potential of materials based on chemical and ecotoxicological tests [22–26]. The proposed global methodology assumes the division of materials into two distinct groups: raw materials (e.g., cement, NA, RA, FA) and construction materials (e.g., concrete). The raw materials group includes materials that are incorporated in cement-based products and is divided into four subgroups: virgin raw materials (VRM); processed raw materials (PRM); recycled and by-product raw materials (RBPRM); and nanomaterials.

A simplified flowchart explaining the methodology adopted to perform the chemical characterization (CC) and ecotoxicological characterization (EC) is presented in Figure 1. Detailed information regarding this methodology can be found in the previous study of the authors [21]. The procedure started with crushing a previously produced source concrete and collecting the remaining RA. Then, the collected RA and other raw materials (FA, cement, NA) were used to produce three new concrete mixes (NAC, C40FA60NA100, C40FA60RA100). The materials' characterization and concrete production are further described in Section 3. The three concrete mixes were then crushed and three types of recycled aggregates (A1, A2, and A3) were produced. After that, the eluates of RA (from the source concrete), FA (raw material), A1, A2, and A3 (from the three concrete mixes) were collected by a leaching procedure and tested for CC and EC (Figure 1) as described in Section 4.

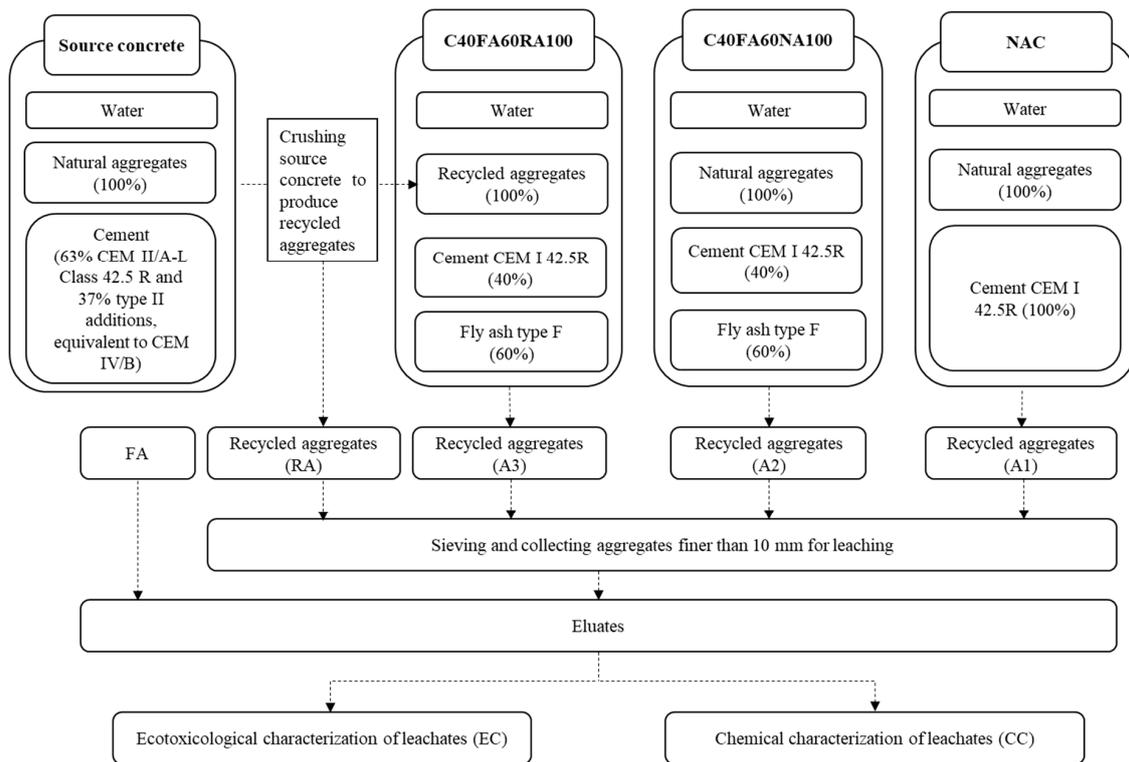


Figure 1. Flowchart of the methodology to perform chemical and ecotoxicological characterizations.

3. Material Characterization and Concrete Compositions

The raw materials chosen to produce concrete mixes include: fine natural aggregates (FNA); coarse natural aggregates (CNA); Portland cement (type CEM I 42.5 R); fine recycled aggregates (FRA); coarse recycled aggregates (CRA); and industrial by-products (FA type F from a Portuguese coal power plant). The materials’ characteristics are presented in Tables 1 and 2. FRA and CRA were initially recycled from a source concrete provided by Unibetão Company (Secil, Lisboa, Portugal) with 63% CEM II/A-L Class 42.5 R and 37% type II additions, equivalent to CEM IV/B. Prior to crushing, the source concrete was cured and kept outside (exposed to air) for 28 days.

Regarding the construction materials to be studied, three concrete mixes (NAC, C40FA60NA100 and C40FA60RA100) were produced, with the incorporation of the mentioned raw materials (Table 3 and Figure 1). NAC was produced with 100% NA and 100% Portland cement, C40FA60NA100 with 40% cement, 60% FA and 100% NA, and C40FA60RA100 with 40% cement, 60% FA and 100% RA in weight.

Table 1. Characterization of cement and FA.

Property		Cement (CEM 42.5 R)	FA (Type F)
f_{ctm} at 28 days (MPa)		10.1	-
f_{cm} at 28 days (MPa)		57.7	-
Specific gravity (g/cm ³)		3.1	-
Residue on 45 μ m sieve (%)		6.2	14.42
Residue on 32 μ m sieve (%)			22.48
Final setting (min)		231.7	
Initial setting (min)		161.1	
Chemical composition (%)	Al ₂ O ₃	5.0	20.9
	CaO	63.5	3.6
	CaO (L)	1.3	
	Cl ⁻	0.0	
	Fe ₂ O ₃	3.3	7.4
	K ₂ O	0.6	1.7
	MgO	1.3	1.0
	Na ₂ O	0.2	1.0
	SiO ₂	19.5	57.8
	SO ₃	3.3	0.6
LOI	2.4		
IR	1.2	3.8	

Legend: f_{ctm} = Flexural strength; f_{cm} = Compressive strength; LOI = Loss on ignition; IR = Insoluble residue; FA = Fly ash.

Table 2. Characterization of natural and recycled aggregates.

Property	FNA		CNA		"Rice Grain"	FRA	CRA
	Coarse Sand	Fine Sand	Coarse Gravel	Fine Gravel			
Bulk density (kg/m ³) [27]	1684	1626	1385	1391	1449	1385	1280
Oven dried density (kg/m ³) [27]	2600	2594	2625	2742	2681	2218	2389
Water absorption (%) [27]	0.5	0.4	1.4	1.2	1.0	8	5
LA abrasion mass loss (%) [28]	-	-	28.6	27.2		-	43
Shape index [29]	-	-	15.6	18.0	17.1	-	25.6

Legend: LA = Los Angeles; FNA = Fine natural aggregates; CNA = Coarse natural aggregates; FRA = Fine recycled aggregates; CRA = Coarse recycled aggregates.

Table 3. Concrete mix design.

Concrete	Binder (%)		Natural Aggregates (%)		Recycled Aggregates (%)	
	Cement	FA	FNA	CNA	FRA	CRA
NAC	100	0	100	100	0	0
C40FA60NA100	40	60	100	100	0	0
C40FA60RA100	40	60	0	0	100	100

Legend: FA = Fly ash; FNA = Fine natural aggregates; CNA = Coarse natural aggregates; FRA = Fine recycled aggregates; CRA = Coarse recycled aggregates.

The produced concrete specimens were cured for 28 days in a dry chamber with relative humidity of $50 \pm 5\%$ and 22 ± 2 °C air temperature. Then, the concrete specimens were fragmented through a jaw crusher in conjunction with a vertical shaft impact crusher. After that, the aggregates were sieved with 10 mm mesh aperture [30]. The three types of aggregates produced from crushing NAC, C40FA60NA100, and C40FA60RA100 were named A1, A2, and A3, respectively. The aggregates recycled from source concrete (named RA) and NAC (named A1) were almost identical, since they were both made with similar NA and cement content. The only differences were in the cement type and curing process of concrete mixes.

4. Testing Methods

The tests performed are summarized in Table 4. The leaching test and further CC and EC of the obtained eluate samples were carried out in the Analytical Laboratory of Instituto Superior Técnico (LAIST, Técnico-Lisboa, Portugal) following recommended guidelines as indicated (Table 4), except for the yeast growth inhibition test, which used an in-house microplate susceptibility test (Table 4).

Table 4. Testing methods.

	Tests	Methodology
Leaching	Production of eluates As, Hg, Sb, Se Ba, Cd, Cr, Cu, Mo, Ni, Pb, Zn Chloride, Fluoride, Sulphate	EN 12457-4 [31] ISO 11885 [32] SMEWW 4110 [33]
Chemical characterization (CC)	Total dissolved solids (TDS) Dissolved organic carbon (DOC) pH Electrical conductivity (ELC)	SMEWW 2540 [34] SMEWW 5310 [35] SMEWW 4500 [36,37] EN 27888 [38]
Ecotoxicological characterization (EC)	Inhibition of <i>Vibrio fischeri</i> luminescence Inhibition of the mobility of <i>Daphnia magna</i> Inhibition of yeast growth	15 to 30 min exposure in static test (ISO 11348-3 [39]) 24 and 48 h of exposure in static test (ISO 6341 [40]) 16 h exposure ([14,21]; microplate susceptibility test)

The leaching test was intended to simulate the behaviour of materials when they come into contact with water as leaching agent under given conditions. This test allows predicting the behaviour of the waste if it is deposited in a landfill or in contact with an ecosystem, assuming this process may occur similarly in the environment. In this study, eluate samples were obtained in accordance with the requirements of EN 12457-4 [31]. The test consists of leaching a portion of the solid raw materials with a liquid/solid ratio of 10 over a period of 24 h at 20 ± 2 °C. Deionized water (ultrapure) through the Millipore system (Merck KGaA, Darmstadt, Germany) was used as a leaching agent, with practically zero electrical conductivity. The pH of this water was close to that of rainwater (pH 5–7). The leaching test according to EN 12457-4 [31] required the materials to be fragmented and sieved to obtain particles finer than 10 mm. The mixture of the obtained solid material with the deionized water was placed in a vessel and mechanically agitated for a period of 24 ± 1 h in a shaking equipment, with orbital motion. Subsequently, the samples were subjected to vacuum filtration through 0.45 µm porosity filter paper and placed in a sterile container at 4 °C until further analysis (CC and EC).

The CC of the eluate samples aimed at classifying the materials in relation to their potential danger. Therefore, the parameters analysed for CC (Table 4) were selected from the ones established in Directive 1999/31/EC [23] and in EC Decision 2003/33/EC [24] that regulate the acceptance of solid waste in landfills; most of them are also present in the French proposal on Criteria on the Evaluation Methods for Waste Ecotoxicity (CEMWE) [25].

The EC of the eluates allows detecting the effect of chemical components that may be present in solution, translating this information into an effect on a test organism used as a bioindicator. In this study, the EC of the samples was made using a battery of three short-term acute toxicity tests with the following test organisms: *Vibrio fischeri*, *Daphnia magna*, and *Saccharomyces cerevisiae* (Table 4), as described before [21]. Briefly, the degree of toxicity was determined from the measurement of the harmful effects caused by various concentrations of each sample, comprising the undiluted eluate sample (100% sample) and a dilution series of it (nominal concentrations: 75%, 50%, 25%, 12.5%, 6.25%, and 3.125%), on the test organisms. For each undiluted or diluted sample, the endpoint measured was bioluminescence upon 30-min exposure for *Vibrio fischeri*, mobility after 24- and 48-h exposure for *Daphnia magna*, or growth after 16-h exposure for the yeast (Table 4). The toxicity index EC_{50} , namely the median-effective concentration of each eluate sample (an eluate concentration that causes a response decrease in the exposed organism population to 50% of that measured in a non-toxic control sample), was determined [21]. These toxicity indexes were further processed following the Toxicity

Classification System (TCS) of Persoone et al. [41] in order to facilitate sorting the samples according to their measured ecotoxicity level. Therefore, the EC_{50} values obtained for each test and eluate sample were converted into toxicity units (TU) as per Equation (1) [41]:

$$TU = (1/EC_{50}) \times 100 \quad (1)$$

The calculated TU values allowed classifying each eluate sample into one of the five ecotoxicity classes defined by Persoone et al. [41], as seen in Table 5. This classification is based on the highest TU value determined for each sample in the battery of tests performed battery (i.e., based on the most sensitive ecotoxicity test).

Table 5. Ecotoxicity classes defined according to the TCS system [41].

Classes	I	II	III	IV	V
Toxicity level	No acute toxicity	Low acute toxicity	Acute toxicity	High acute toxicity	Very high acute toxicity
TU	≤ 0.4	$0.4 < TU \leq 1.0$	$1.0 < TU \leq 10$	$10 < TU < 100$	$TU \geq 100$

5. Results and Discussion

The application of the proposed methodology to classify the materials under study in terms of hazard regarding the acceptance of solid waste at landfills and ecotoxicological potential allowed obtaining the results that are presented in Table 6. NA are considered VRM, which were previously classified as inert raw materials with no evidence of ecotoxicity (NOE) (Table 6 and [21]); this result can be supported by the study of Barbudo et al. [42].

Table 6. Classification of the raw materials and of the concrete mixes according to the EC Decision 2003/33/EC (pursuant to Directive 1999/31/CE), the document of the CEMWE French proposal and the TCS system.

Materials		Chemical Characterization		Ecotoxicological Characterization		
		Directive 1999/31/EC [23] EC Decision 2003/33/EC [24]		CEMWE [25]		TCS (Persoone et al. [41])
		Class	Parameter	Class	Parameter	
Raw materials	NA	Inert	-	NOE	n/a	n/a
	Cement	-	-	NOE	n/a	n/a
	RA	Non-dangerous	TDS	NOE	n/a	Class III
	FA	Hazardous	Se, Cr, Mo	NOE	n/a	Class III
Construction materials	A1	Non-dangerous	TDS	NOE	n/a	Class III
	A2	Non-dangerous	TDS, Cr, Mo	Ecotoxic	<i>D. magna</i>	Class IV
	A3	Non-dangerous	TDS, Cr, Mo	Ecotoxic	<i>D. magna</i>	Class IV

Legend: NA = Natural aggregates; RA = Recycled aggregates; FA = Fly ash; A1 = Aggregates recycled from NAC; A2 = Aggregates recycled from C40FA60NA100; A3 = Aggregates recycled from C40FA60RA100; TDS = Total dissolved solids; NOE = No evidence of ecotoxicity; n/a = Not applicable; *D. magna* = *Daphnia magna*; Class III = Acute Toxicity; Class IV = High Acute Toxicity.

According to the European regulation for Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) [43], Portland cement is considered a well-defined mixture (Table 1); it is in the RBPRM group of raw materials. The existence of a safety data sheet (SDS) for Portland cement shows that this mixture meets the criteria for the classification of hazardous substances set out in the CLP regulation [44]. According to the information provided in the SDS, this material could be classified as non-dangerous for the aquatic environment [44]. Thus, it was not considered necessary to perform the CC and EC of cement, and it may be classified as NOE raw material (Table 6). This result is consistent with reports of Hillier et al. [17] and Gwenzi and Mupatsi [19].

RA prepared from source concrete also belong to the group of RBPRM. In this study, the CC and EC of eluate sample obtained from RA were performed (results described in Sections 5.1 and 5.2, respectively). The original RA constituents were the following raw materials: water, NA and cement (CEM II/AL 42.5R), which already have been classified [2]. Therefore, in the particular case in which RA were obtained from the crushing of factory-produced concrete with the desired characteristics (Figure 1), and its composition is fully known (Table 2), it can be assumed that the classification of this raw material can be made from the results obtained for the eluates of the aggregates produced from the reference concrete. The compositions are identical, and both the CEM II/AL 42.5R and the CEM I 42.5R cements (Figure 1) are regulated by the same SDS.

A SDS is not available for the FA used in this study; this type of raw material can be considered as potentially non-hazardous (unknown or variable composition substances) and expected to cause no or low ecotoxicological risk to the aquatic environment [21]. However, a REACH product information sheet is available for “coal ashes (residues)” under the REACH Registration number 01-2119491179-27-0012, which reports measurable no-observed-effect-concentration (NOEC) values for coal ashes towards representative organisms of freshwater, marine and soil ecosystem. Therefore it was found important to examine eluate samples obtained from the FA used in the present study in terms of CC and EC; the results were reported for the first time in [21] and are herein compared with the CC and EC data for the concrete mixes under study (in Sections 5.1 and 5.2, respectively).

The incorporation of RA and FA in cementitious building materials, respectively replacing NA and cement, may have environmental benefits because they avoid landfilling of waste considered hazardous such as FA.

5.1. Chemical Characterization (CC) of RA, FA, A1, A2 and A3 Eluate Samples

The results for the non-metallic and metallic parameters from the CC of the eluate samples obtained from of the raw materials (RA and FA) and concrete mixes (A1, A2, and A3) under study are presented in Table 7.

Table 7. Data of the chemical characterization of the eluate samples.

Parameters		RA	FA	A1	A2	A3
Non-metallic parameters	pH at 22 °C	12.5	11.8	12.5	12.4	12.1
	ELC (µS/cm)	6950	1672	6950	4300	3560
	DOC (mg/kg)	88	23	88	11	100
	TDS (mg/kg)	17,000	6450	17,000	12,000	9200
	Chloride (mg/kg)	15	<30	15	19	34
	Fluoride (mg/kg)	<10	<10	<10	<10	<10
	Sulphate (mg/kg)	<30	4400	<30	<30	37
Metallic parameters (mg/kg)	As	<0.4	<0.4	<0.4	<0.4	<0.4
	Ba	4.0	4.6	4.0	9.0	8.0
	Cd	<0.1	<0.1	<0.1	<0.1	<0.1
	Cr	<0.5	2.5	<0.5	0.5	0.7
	Cu	<1.0	<0.5	<1.0	<1.0	<1.0
	Hg	<0.2	<0.2	<0.2	<0.2	<0.2
	Mo	<0.3	10.0	<0.3	0.6	1.00
	Ni	<0.4	<0.4	<0.4	<0.4	<0.4
	Pb	<0.5	<0.5	<0.5	<0.5	<0.5
	Sb	<0.4	<0.4	<0.4	<0.4	<0.4
	Se	<0.2	4.0	<0.2	<0.2	<0.2
Zn	<0.5	<0.5	<0.5	<0.5	<0.5	

Legend: ELC = Electrical conductivity; DOC = Dissolved organic carbon; TDS = Total dissolved solids; RA = Recycled aggregates; FA = Fly ash; A1 = Aggregates recycled from NAC; A2 = Aggregates recycled from C40FA60NA100; A3= Aggregates recycled from C40FA60RA100.

Regarding the non-metallic parameters, it was found that all the eluate samples were highly alkaline, with pH values varying in the following order: FA (pH = 11.8) < A3 (pH = 12.1) < A2 (pH = 12.4) < A1 = RA (pH = 12.5) (Table 7). The lower pH value of FA was compared with the results of Moreno et al. [45] and Tsiridis et al. [10]. The value from our study was closer to the mean value of Tsiridis et al. [10], possibly because the leachates were produced based on the same standard procedure [31]. The pH values of RA, A1, A2 and A3 eluates are common in this type of construction materials; they are close to concrete's pH (approximately 12.5) and are slightly higher than that of FA (Table 7). These high pH values may be due to the presence of carbonates, oxides and hydroxides formed during combustion processes [26]. On what concerns the concrete mixes, it is worthy to note that the eluate pH slightly decreased (about 0.4 pH units) with the incorporation of 60% of FA and 100% of RA (comparing A3 to A1; Table 7). However, as the pH value of FA depends on the sulphur content of the source coal, it can vary between 4.5 and 12.0 [46,47]. Therefore, this study suggests that the incorporation of FA in concrete may lead to a decrease of the pH of the respective eluates, possibly contributing to a lower possible negative impact of alkaline eluate runoff (for instance, due to heavy raining) into aquatic environments, in landfills and/or during building service.

Regarding acceptability as solid waste in landfills, the raw materials (FA and RA) and concrete mixes (A1, A2 and A3) cannot be considered inert because the TDS concentration values measured in the respective eluate samples (Table 7) are higher than the limit value of 4000 mg/kg defined in the EC Decision 2003/33/EC [24] for inert waste. However, based solely on TDS measurements, they can be considered as non-hazardous waste (Table 6) since the TDS values are lower than the limit values (60×10^3 mg/kg) defined for non-hazardous waste in the same EC Decision [24].

Regarding the concentration of sulphate (SO_4^{2-}), a sulphur (VI) compound, FA showed the highest value among all the materials (4400 mg/kg), much higher than the limit values defined in the EC Decision 2003/33/EC [24]. Therefore, in terms of the sulphate concentration, it is advisable to use FA in concrete because the solidification process caused by the hydration of this binder is effective on retaining heavy metals, namely sulphate. The sulphate concentration in the remaining samples was close to 30 mg/kg, much lower than the limit values [24].

On what concerns the metallic parameters analysed in the eluate samples (Table 7), of the studied elements, Cd, Hg, Ni, and Pb belong to the list of priority substances in the field of water policy and Cd and Hg are considered priority hazardous substances [48]. From this set of metals evaluated in the RA sample, only Ba presented a concentration value above the limit of quantification (4.0 mg/kg) (Table 7). RA can be considered a non-hazardous waste because the concentrations of all the elements in the respective eluate (Table 7) were lower than the limit values established for non-hazardous waste the EC Decision 2003/33/EC [24] (Table 6). On the contrary, in the FA eluate sample, Ba (4.6 mg/kg), Cr (2.5 mg/kg), Mo (10 mg/kg) and Se (4.0 mg/kg) were detected at concentrations above the quantification limits (Table 7). The concentration values of metals leached from the FA in this study were close to the average values obtained by Moreno et al. [45] (determined based on 23 different samples of FA). In the present work, among these metal concentrations, only Se is above the limit value (0.5 mg/kg) defined for non-hazardous waste in EC Decision 2003/33/EC [24] and thus FA is classified as potentially hazardous for the environment (Table 6 and [21]).

For the eluate samples from A1, A2, and A3, only Ba, Cr, and Mo showed concentrations above the minimum detection limit (Table 7). A1 eluate presented a Ba concentration (4.0 mg/kg) lower than that of FA (4.6 mg/kg); the remaining metal parameters showed concentration values lower than the detection limit (Table 7). For the A2 sample, the Ba concentration (9.0 mg/kg) was approximately twice the one recorded in the FA sample, while the concentrations of Cr and Mo were 80% and 94% lower in A2 compared with FA, respectively (Table 7). A similar trend was observed for the A3 sample (Table 7). It is worthy of note that the concentration of Se for all three concrete samples was zero or below the detection limit (Table 7). Importantly, the results show that all the metals concentrations in the A1, A2 and A3 eluates were lower than the limit values defined for non-hazardous waste in the landfill EC Decision [24].

Overall, based on the results obtained from the CC, the samples RA, A1, A2, and A3 were classified as non-dangerous and the sample FA as potentially hazardous regarding possible contamination of aquatic environments associated to possible leaching of metals from waste disposal in landfills (Table 6).

5.2. Ecotoxicological Characterization (EC) of RA, FA, A1, A2 and A3 Eluate Samples

The results of the EC are presented in Table 8. Despite the alkaline nature and slightly different pH of the tested eluates samples (Table 7), no corrections were made to their pH values in toxicity testing, since it was intended to simulate environmental exposure conditions as close as possible to the real ones. Therefore, the ecotoxicity indexes and TU values presented in Table 8 combine the contributions of possible harmful effects associated not only with the chemical components present in the eluate samples but also with their alkaline pH values.

Table 8. Ecotoxicological characterization results.

Materials	pH	Bacterium <i>Vibrio fischeri</i>		Crustacean <i>Daphnia magna</i>			Yeast <i>Saccharomyces cerevisiae</i>		
		EC ₅₀ (%) [30 min]	TU	EC ₅₀ (%) [24 h]	TU [24 h]	EC ₅₀ (%) [48 h]	TU [48 h]	EC ₅₀ (%) [16 h]	TU
RA	12.5	>100	<1	18.8	5.3	14.6	6.8	19.8	5.1
FA	11.8	49.3	2.0	30.8	3.2	30.8	3.2	>100	<1
A1	12.5	>100	<1	18.8	5.3	14.6	6.8	19.8	5.1
A2	12.4	>100	<1	6.8	15	5.5	18.8	30.2	3.3
A3	12.1	>100	<1	13.6	7.4	7.7	12.9	40.7	2.5

Legend: RA = Recycled aggregates; FA = Fly ash; A1 = Aggregates recycled from NAC; A2 = Aggregates recycled from C40FA60NA100; A3 = Aggregates recycled from C40FA60RA100; TU = Toxicity Units; EC₅₀ = median-effective concentration for the endpoint in each test.

The eluate sample of RA (equal to A1) did not cause inhibition of *Vibrio fischeri* luminescence. Comparatively, the toxicity of the same samples to *Daphnia magna* and *Saccharomyces cerevisiae* test organisms was higher (Table 8). These results suggest that the bioluminescent bacterium was not sensitive to exposure to the RA (or A1) sample. On the contrary, the crustacean and the yeast tests presented similar results between them, with TU values between 5.3 and 6.8 in the *Daphnia magna* toxicity test (24 h and 48 h exposure, respectively) and equal to 5.1 in the yeast toxicity test (16 h exposure) (Table 8). These TU values higher than 1 but considerably lower than 10 (TCS system [41]; Table 5) in both toxicity tests with RA (or A1) indicate potentially low acute ecotoxicity (class III) of these materials (Table 6).

Regarding the FA eluate sample, *Vibrio fischeri* bioluminescence and *Daphnia magna* mobility were both moderately affected by this sample, contrary to the yeast growth which was not inhibited upon exposure to the FA sample (Table 8 and [21]). The results reported in the study of Tsiridis et al. [10,49] indicated *Daphnia magna* among the diverse test organisms they used (bacteria, microalga, crustacean, and rotifer), to be the most sensitive test organism in the evaluation of the ecotoxicity of coal FA leachates [10,49]. The authors attributed this result to the lower tolerance of this invertebrate to the presence of high Cr concentration in the samples [49]. This study also revealed that the presence of Cu, Ni, and Zn in the leachate samples resulted in a significant bioluminescence inhibition of *Vibrio fischeri*. In our study, the concentrations of these metals in all eluate samples are very low (Table 7), so the resulting effect is expected to be low. The TU values much lower than 10 (TCS system [41]; Table 5) in both *Daphnia magna* and *Vibrio fischeri* toxicity tests (TU = 3.2 and 2, respectively; Table 8), suggest very low acute ecotoxicity (class III) of FA eluate (Table 6).

The eluate samples of A2 and A3 did not cause inhibition of *Vibrio fischeri* bioluminescence. However, both the crustacean mobility and the yeast growth were considerably affected by the A2 and the A3 samples (Table 8). In both cases, the comparison of the respective TU values indicated that

the *Daphnia magna* toxicity test (TU [24 h] = 7.4 and 15 for A3 and A2, respectively) was much more sensitive than the yeast one (TU [16 h] = 2.5 and 3.3, respectively) (Table 8). Based on the TU values > 10 for the most sensitive ecotoxicity test used, i.e., 48 h mobility for *Daphnia magna* (Table 8), the samples A2 and A3 were classified as moderate-highly toxic (class IV) (Table 6). Comparatively, both the A3 and A2 samples were found to exert higher levels of ecotoxicity towards the freshwater model crustacean than the A1 sample, and the order of potential ecotoxicity is: A2 > A3 > A1 (Tables 6 and 8).

Given the results obtained, there was also no evidence to classify the eluate samples of RA, FA and A1 as ecotoxic (Table 6), since the results of CC and EC for these materials (Tables 7 and 8) comply with the limit values established in the French proposal CEMWE [25]. However, the results obtained for samples A2 and A3 allow classifying these materials as potentially ecotoxic based on the fact that the limit value of 10% for 48 h mobility EC_{50} in *Daphnia magna* (corresponding to TU = 10 in Table 5) defined in the French proposal CEMWE [25] was exceeded (Table 6).

There are reports by other authors of a considerable sensitivity of *Daphnia magna* invertebrates when suspended in alkaline media (pH values as high as 11.3) [26]. In our work, despite the high pH values of all the eluate samples tested (Table 8), pH is not anticipated to be the only relevant factor contributing to the negative effects of the samples of this model invertebrate. This is because eluates from RA (A1) with a pH value of 12.5 were found much less toxic to the crustacean than the A2 and A3 eluates with pH values equal to 12.4 and 12.1, respectively (Table 8). Therefore, other factors, for instance the release of chemical compounds, including metals, from these materials into water due to leaching, may be influencing the biological response of the test-organism to the eluates' samples, as reported by others [13,19].

Of the three short-term ecotoxicity tests used (with *Vibrio fischeri*, *Daphnia magna*, and *Saccharomyces cerevisiae*), the most sensitive one in analysing the ecotoxicity of cement-based raw and construction materials was the standard acute mobility test with the freshwater crustacean *Daphnia magna*. On the contrary, the bioluminescent bacterium test was the least sensitive to all eluates' samples, while the microplate susceptibility test with *Saccharomyces cerevisiae* showed an intermediate response (except for the FA eluate sample). In this study, the yeast-based test is proposed as a suitable test for preliminary screening of the potential ecotoxicity of raw and construction materials. Even though a relatively low sensitivity of the yeast test, compared with the *Daphnia magna* one, should be recognized, the yeast test provides some relevant advantages as test system, namely: sparing of higher eukaryotes in toxicity testing (required by regulators, e.g., REACH); meaningfulness of data for eukaryotic microorganisms that provide relevant services in ecosystems; simplicity; low-cost; small-scale (96-well microplate format) and reproducibility [21].

The potential leaching of metals and other chemicals from FA, A2 and A3 was very low for almost all analysed metals, including the ones in the European Union list of priority substances in the field of water policy (e.g., Cd, Hg, Pb, and Ni) [44]. Nevertheless, regarding landfill deposition and based on the CC alone, FA eluate was classified as hazardous (due to the Se level) while the eluates of A2 and A3 were considered non-hazardous (due to Mo and TDS levels) (Table 6). On the contrary, based on EC, the former was NOE or class III (according to CEMWE or TCS, respectively) while the latter were classified as class IV based on ecotoxicity towards *Daphnia magna* (Table 6). These changes can be due to possible differences in the chemical composition of the eluates and/or in other parameters not analysed in this work. Based on the data presented in this work and on other studies [19,26], it can also be suggested that the alkaline pH of eluates from FA (pH 11.8) and concrete (pH 12.5 and 12.4 for A1 and A2, respectively) may be relevant in this respect and may contribute to possible environmental risks. Such risks can be especially important if leachates or eluates from concrete or FA are produced in buildings in service (e.g., due to rain events) and/or in landfills and can reach freshwater ecosystems leading to water alkalisation. Therefore, results obtained in this study highlight the importance of integrating data from CC and EC of materials' eluate samples aiming at assessing the possible environmental risk of the construction materials, as suggested by other authors [26,50].

The incorporation of raw materials that have a low ecotoxicity level (e.g., FA, RA) in construction materials can however generate construction materials leading to eluates with a significantly higher toxicity level. For example, A1, coming from a concrete with 100% NA and 100% cement, has a toxicity level lower than that of A2 and A3 where natural aggregates and Portland cement were partially replaced with non-conventional RA and FA. In fact, it seems relevant that although FA has a low ecotoxicological potential (Table 6), when this raw material was incorporated in concrete mixes in a given quantity (60% in the present study) it did not contribute to diminish the potential ecotoxicity of the respective eluates.

The literature states that the cement-based construction materials that raise the most concerns in terms of ecotoxicological risk are those that incorporate RA. However, the incorporation of by-products (FA) in such materials may also contribute to some non-negligible environmental risk. These raw materials have a variable chemical composition that is often unknown.

6. Conclusions

From the application of a methodology proposed in a previous study [21] to three concrete mixes (A1, A2, A3), in order to compare the ecotoxicological potential of raw materials (fly ash—FA, recycled aggregates—RA) and cement-based construction materials, the following conclusions were drawn:

- Taking into account only the chemical characterization (CC) of the eluates and the comparison of the results obtained with limit values established in the landfill waste EC Decision 2003/33/EC [24], the concrete mixes formulated with raw materials classified as hazardous (FA) may be classified as non-hazardous materials (A2 and A3).
- Materials with a high ecotoxicological potential, namely samples A2 and A3, can be formulated from raw materials with evidence of lower ecotoxicity (FA and RA). This result is corroborated by TCS, which showed that the ecotoxicity degree of construction materials formulated with ecotoxic raw materials was greater than that of their raw materials; an increase in toxicity occurs from the raw materials (Class III) to the construction materials (Class IV). Thus, the incorporation of 100% RA and 60% FA in concrete may result in an adverse effect on human health and on the environment.
- The incorporation of high quantities of FA with an extremely high sulphate concentration (4400 mg/kg) in concrete reduced the concentration to 30 mg/kg for the samples A1, A2, and A3. The concentration of chlorides was low in FA, but increased slightly when incorporated in concrete.

In this study, the importance of assessing the possible environmental risks of the incorporation of non-conventional raw materials in cement-based construction materials was demonstrated. The chemical and ecotoxicological characterization of materials' eluate samples can complement environmental life cycle assessment studies and contribute to achieving construction sustainability.

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