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Research article

Eco-toxicity assessment of industrial by-product-based alkali-activated binders using the sea urchin embryogenesis bioassay

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ABSTRACT

New cement-based materials such as alkali-activated binders (AABs) or geopolymers allow the incorporation of waste or industrial by-products in their formulation, resulting an interesting valorization technique. Therefore, it is essential to inquire about the potential environmental and health impacts throughout their life cycle. In the European context, a minimum aquatic toxicity tests battery has been recommended for construction products, but their potential biological effects on marine ecosystems have not been considered. In this study, three industrial by-products, PAVAL® (PV) aluminum oxide, weathered bottom ash (WBA) resulting from incinerator bottom ash and glass cullet recycling waste (CSP), were evaluated as precursors in the AAB formulation from an environmental point of view. To determine the potential effects on marine environment caused by the leaching of contaminants from these materials into seawater, the leaching test EN-12457-2 and an ecotoxicity test using the model organism sea urchin *Paracentrotus lividus* were conducted. The percentage of abnormal larval development was selected as endpoint of the toxicity test. Based on the results obtained from the toxicity tests, AABs have less damaging impact (EC_{50} values: 49.2%–51.9%) on the marine environment in general than raw materials. The results highlight the need to stablish a specific battery of toxicity tests for the environmental assessment of construction products on marine ecosystem.

1. Introduction

The construction sector is a natural engine for generating wealth, but it has a significant impact on the environment (Yu et al., 2022). Currently, in the materials industry, circular economy practices drive the reuse of waste to reduce natural resource depletion, disposal of industrial waste, and in some cases, energy consumption, as well as the reduction of polluting gas emissions (Grdic et al., 2020). Thus, one of the great challenges in the construction industry is the use of new cement-based materials such as alkali-activated binders (AABs) or geopolymers. AABs essentially encompass any binder system derived from the reaction between an alkali metal and a solid with some silicon content (silicate or aluminosilicate). In recent years, several studies conducted on the synthesis of AABs as a technique for incorporating waste or industrial by-products into their formulations have emerged (Ji and Pei, 2019; Zhao et al., 2021). The main goal of AAB manufacturing is the use of residual materials from a variable source, promoting sustainable criteria such as the circular economy and the reduction of natural resource extractive activity (Robayo-Salazar et al., 2018). In this sense, residues with significant alumina and silica content can be used as precursors for AAB formulation, regardless of their alkali content (Palomo et al., 2021). The specific waste materials used in the production of AABs may vary depending on their availability and properties in a given location. In this study, three industrial by-products were evaluated as precursors in the AAB formulation from an environmental point of view: (i) PAVAL® (PV) aluminum oxide by-product generated during the recovery of metallic aluminum from salt slag in the secondary aluminum refining process (IECA, 2014), (ii) weathered bottom ash (WBA) resulting from the maturation treatment of the incinerator bottom ash (IBA) generated in waste-to-energy (WtE) plants, and (iii) glass cullet

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Table 1
Chemical composition of the major and minor elements in the raw materials used for the alkali-activated binder formulations. MK: Metakaolin; PV: PAVAL®; WBA: Weathered Bottom Ash; CSP: Glass Recycling Waste.

(wt.%)	SiO_2	CaO	Al_2O_3	Na_2O	K_2O	Fe_2O_3 [b]	MgO	TiO_2	Cl^-	SO_3	LOI [a]
MK	55.00	0.30	40.00	0.80	0.80	1.40	0.30	1.50	–	–	1.00
PV	7.93	2.24	61.02	3.22	0.68	2.65	4.73	0.80	–	–	15.71
WBA	52.08	20.72	6.35	3.38	2.09	4.12	2.43	0.65	0.54	1.07	6.10
CSP	70.78	9.37	4.81	11.15	0.94	0.57	1.61	0.13	–	–	0.99

[a] LOI: Loss on ignition at 1000 °C.
[b] The content of iron is expressed as total iron oxide, calculated by stoichiometry from iron signal, as usual, is reported for results from XRF spectrometry analysis.

recycling waste known as ceramic, stone, and porcelain (CSP). IBA is classified in the European context as a hazardous (EWC 190111*) or non-hazardous waste (EWC 190112) depending on its concentration of hazardous substances (Maldonado-Alameda et al., 2021), while CSP contains approximately 84 wt% glass that has not been able to be classified by optical sorting equipment during recycling (bottlenecks and bottoms) (Giro-Paloma et al., 2021). The composition of these by-products and residues makes them suitable raw materials for obtaining AABs. These novel formulations have been developed in previous works by the authors (Maldonado-Alameda et al., 2020, 2021).

To estimate the potential environmental impacts of the new construction materials, the chemical characterization of the potential hazard of the waste or industrial by-products is not enough. Chemical transformations, which take place under high alkalinity conditions when waste-based binders are formulated, as well as the conditions of use of the construction material, affect the leaching behavior and, consequently, the potential toxicity to the environment (Kobetičová and Černý, 2017; Ribé et al., 2014). Therefore, when waste is used in construction materials, it is essential to inquire about the potential environmental and health impacts in the different stages of their life cycle, such as the construction and demolition stages or service life and disposal (Maia et al., 2022; Rodrigues et al., 2017). In the European context, a test battery has been developed for the ecotoxicological characterization of eluates from construction products for regulatory purposes and voluntary initiatives by manufacturers or eco-labels (CEN/TR 17105:2017) (CEN, 2017). This technical report proposes four aquatic tests using different species along the trophic chain (algae, *Daphnia*, luminous bacteria, and fish eggs) to evaluate their impact on freshwater environments. These tests are particularly relevant for complex products that cannot be easily evaluated by chemical analysis, such as organic/polymeric products or inorganic products with organic additives (Gartiser et al., 2017a; Heisterkamp et al., 2019). Although this minimum test battery for aquatic toxicity tests has been recommended for construction products for outdoor use, the potential biological effects of these products on marine ecosystems (e.g., construction in port areas and artificial reefs) have not yet been considered.

Bioassays of the embryonic and larval stages of marine invertebrates are widely used to assess the quality of marine environments (Beiras et al., 2021; Gopalakrishnan et al., 2008; His et al., 1999). Sea urchins are among the most common model organisms for ecological and toxicological studies, owing to their abundance in the marine ecosystem, their essential role in coastal ecosystem maintenance, limiting algal biomass, and serving as food for other predators (Sevillano-González et al., 2022; Steneck, 2013), as well as for the simplicity and standardization of the tests using this species (Garmendia et al., 2009). The sea urchin *Paracentrotus lividus* has been used as an ecological indicator of marine pollution as its first stages of embryonic development make it a sensitive model for a variety of pollutants, single or mixed contaminants, or multiple stressors in natural ecosystems (Gambardella et al., 2021), especially for metal contamination (Bonaventura et al., 2018; Fernandez and Beiras, 2001; Fichet and Miramand, 1998; Manzo et al., 2010; Morroni et al., 2018). In addition, this test has been regularly used to assess complex environmental matrices, such as contaminated dredged marine sediments (Bonaventura et al., 2021; Carballeira et al., 2012; Khosrovyan et al., 2013; Volpi Ghirardini et al., 2005). This species has also recently been used in toxicity tests of artificial reefs (Santos et al., 2023), wastewater treatment plant effluents (Mijangos et al., 2020), emerging contaminants (Asnicar et al., 2020), microplastic-associated contaminants (di Natale et al., 2022; Trifuoggi et al., 2019) and leachates of microplastic particles (Oliviero et al., 2019; Rendell-Bhatti et al., 2021).

This study aimed to assess the potential toxicity of AABs formulated with PV, WBA, and/or CSP as precursors using the sea urchin *P. lividus*. To date, only a few studies have used an ecotoxicological approach to assess the potential toxicity of construction products using aquatic bioassays (Bandow et al., 2018; Gartiser et al., 2017b; Maldonado-Alameda et al., 2021; Rodrigues et al., 2020; Santos et al., 2023) and only inter-laboratory levels (Heisterkamp et al., 2021). The novelty of this study lies in the application of the sea urchin aquatic bioassay as an environmental tool to evaluate the potential risks of these innovative construction materials in marine environments. Therefore, it was hypothesized that this toxicity test could be a suitable tool for assessing the potential effects of these materials in the marine environment. Furthermore, the integration of the results obtained from the chemical characterization of leachates and sea urchin embryogenesis tests is expected to provide a new perspective for determining optimal AAB formulations based not only on technological properties, but also on their environmental behavior.

2. Materials and methods

2.1. Selection of materials

Various materials have been used as precursors to formulate AABs. Commercial metakaolin (MK) powder provided by the AGS Mineraux Company (Clerac, France) was used to formulate AA-MK as a reference AAB. Furthermore, three different secondary resources were used as precursors: an industrial by-product obtained during the melting process of secondary aluminum PAVAL® (PV) provided by BEFESA, S.A. (Spain), weathered bottom ash (WBA) collected from a waste-to-energy plant located in Tarragona (Spain) and supplied by VECSA company, and a residual fraction of glass cullet recycling (CSP), a sample collected from a CSP separation system in a glass recycling plant by Daniel Rosas, S.A. company located in Barcelona (Spain). In terms of alkaline activators, a commercial Na_2SiO_3 solution with a weight ratio of 3.22 (26.44 wt% of SiO_2 and 8.21 wt% of Na_2O) and a density of 1.37 g cm^{-3} was supplied by Scharlab (Spain). To prepare the NaOH solutions, pellets (ACS–ISO–for analysis) provided by Labbox (Spain) were used.

2.1.1. Material precursor preparation

The following steps were carried out to prepare the secondary materials as alkali-activated precursors. First, 5 kg of PV, WBA, and CSP were separated by quartering to obtain a representative sample of each. Then, after drying the quartered samples, to improve the reactivity of precursors, the PV, WBA, and CSP were crushed and ground using a Jaw Crusher RETSCH BB 50 and Vibratory Disc Mill RETSCH RS20 until a powder particle size of 80 μm was obtained. In addition, a metal magnet (Nd; 0.485 T) was passed over the ground samples (approximately 1 cm

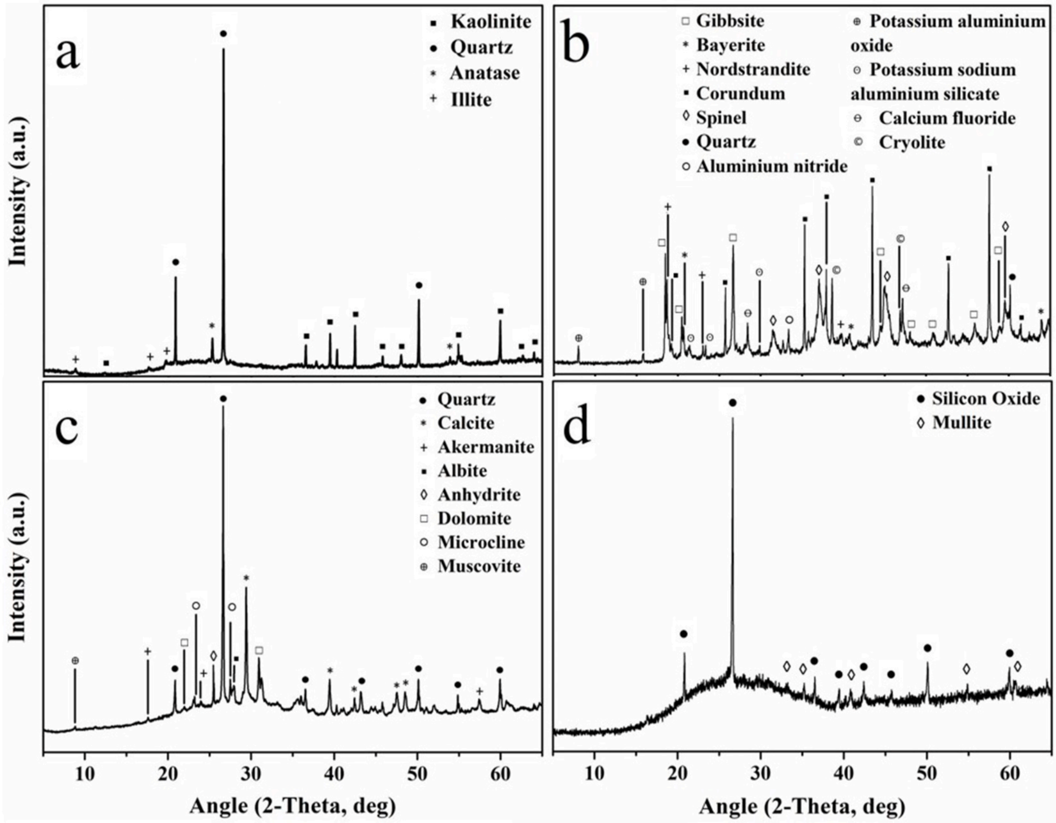

Fig. 1. XRD patterns of raw materials (a) MK: Metakaolin; (b) PV: PAVAL®; (c) WBA: Weathered Bottom Ash; (d) CSP: Glass Recycling Waste.

Table 2
Mixture proportions of powdered precursors (MK: Metakaolin; PV: PAVAL®; WBA: Weathered Bottom Ash; CSP: Glass Recycling Waste) and alkaline activators (sodium hydroxide and sodium silicate solutions) for preparation of alkali-activated binders (AA-MK. AA-WBA/PV and AA- CSP/PV).

Binders	Precursors (S)				Alkaline activators (L)				L/S ratio
	MK[a]	PV[a]	WBA[a]	CSP[a]	NaOH 8 M[a]	NaOH 6 M	NaOH 4 M[a]	Na_2SiO_3[a]	
AA-MK	100	–	–	–	68	–	–	66	1.3
AA-WBA/PV	–	2	98	–	–	20	–	80	0.6
AA-CSP/PV	–	5	–	95	–	–	50	–	0.5

[a] wt. % respect to the total solid.

away from the sample) to remove the magnetic particles.

Elemental X-Ray Fluorescence analysis of the raw materials was carried out using a Panalytical Philips PW 2400 sequential X-ray spectrophotometer equipped with UniQuant® V5.0. The main oxide elements of the precursor materials obtained by X-ray fluorescence (XRF) are listed in Table 1. The MK mainly consisted of SiO_2 (55%) and Al_2O_3 (40%). The WBA mainly consisted of SiO_2 (52%) and CaO (20,72%). PV, which comes from the aluminum industry as mentioned above, is rich in Al_2O_3 (61%). Finally, the CSP, as glass waste, mainly consisted of SiO_2 (70,72%), Na_2O (11.15%), and CaO (9.37%). In all cases, these secondary raw materials contained large amounts of SiO_2 and/or Al_2O_3, which are essential for AAB formulation.

Fig. 1 shows the mineralogical analysis by X-ray diffraction (XRD) performed on the different raw materials using a Bragg–Brentano Siemens D-500 powder diffractometer device with Cu Kα radiation. The XRD pattern analysis of MK (Fig. 1a) revealed the presence of kaolinite ($Al_2Si_2O_5(OH)_4$), quartz (SiO_2), and anatase (TiO_2).

Halloysite ($Al_2Si_2O_5(OH)_4$) and illite ((K,H_3O) $(AlMgFe)_2(Si,Al)_4O_{10}$) are the main crystalline phases. Fig. 1b depicts the PV XRD pattern, where some aluminum-based compounds, mainly alumina (Al_2O_3), spinel ($MgAl_2O_4$), and gibbsite ($Al(OH)_3$), were identified. The mineralogical analysis conducted on WBA (Fig. 1c) demonstrated the presence of silicon, aluminum, and calcium-bearing compounds such as quartz (SiO_2), calcite ($CaCO_3$), albite ($NaAlSi_3O_8$), and dolomite ($CaMg(CO_3)_2$). In the CSP XRD pattern (Fig. 1d), quartz (SiO_2) and mullite ($Al_6Si_2O_{13}$) were detected as the main crystalline phases. Notably, the halo between 20° and 35° shown in MK, WBA, and CSP reveals the amorphous nature of these raw materials, which is crucial for their alkaline activation (Occhipinti et al., 2020).

2.1.2. Alkali-activated binder preparation procedure

The formulations of the studied AABs are shown in Table 2, indicating the precursors (referred to as solid, S) and alkaline activator solutions (referred to as liquid, L) ratios as well as the liquid/solid ratio (L/S). An alkali-activated metakaolin binder (AA-MK) was used as the reference material. AA-WBA/PV is the reference name for the binder formulated through the alkaline activation of WBA and PV. Finally, AA-CSP/PV is the reference name for the binder formulated by CSP and PV as raw material precursors. The preparation was initiated by mechanically stirring the Na_2SiO_3 and NaOH solutions (except for AA-CSP/PV) in a plastic beaker. The precursors were then gradually added to the alkaline activator solution for 2 min at 500 rpm to promote the

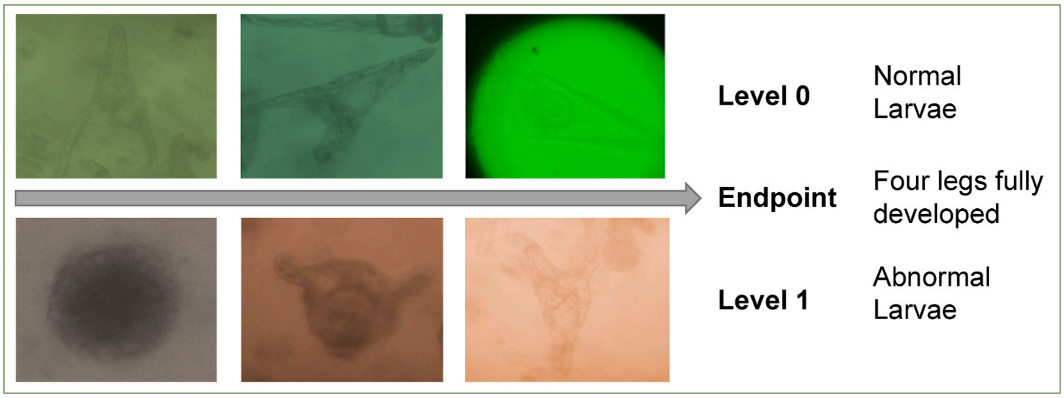

Fig. 2. Sea urchin larval toxicity levels proposed to assess raw materials precursors and AABs. Adapted from Carballeira et al., (2012).

dissolution of the reactive phases in the alkaline media. The mixture was then stirred for 3 min at 750 rpm. The pastes were poured into 40 × 40 × 160 mm prismatic molds and vibrated for 30 s. The molds were then introduced into plastic bags and sealed for three days to avoid water loss. Next, the binders (AA-MK and AA-WBA/PV) were cured in a climate chamber at 20 ± 1 °C and relative humidity of 95 ± 5%. The curing conditions for AA-CSP/PV were 40 ± 1 °C and relative humidity of 10 ± 5%. The specimens were unsealed and demolded after three days and kept in a climate chamber under the same conditions until testing (28 days).

2.2. Leaching test

The potential leaching of the elements in the solid samples was determined using the batch leaching test EN 12457–2 (CEN, 2003), with some modifications. First, the materials were crushed where necessary to obtain a particle size of less than 4 mm. The test was conducted for 24 h at a liquid/solid ratio (L/S) of 10 L kg^{-1} of dry matter at room temperature. In this study, clean filtered (0,45 μm) seawater (pH 7.88 and 31‰ salinity) was used as the leaching agent instead of deionized water to carry out the subsequent marine bioassay. In addition, a control treatment using only a leaching agent (clean filtered seawater) was performed. The leachates obtained were filtered (0,45 μm), homogenized, and separated into two samples. The first sample, used to conduct the chemical analysis, was acidified to a pH of 1.5–2.0 with HCl (approximately 1 μL per 1 mL of the sample) and stored at 4 °C before chemical analysis. A sea urchin embryogenesis test was performed on the second sample. The test was performed in duplicate to reduce the deviation caused by dealing with heterogeneous samples.

2.3. Chemical analysis of leachates

Samples for metal analysis were pre-concentrated using a liquid-organic extraction method with APDC/DDDC22 and analyzed using ICP-MS (PerkinElmer ELAN DRC-e). The accuracy of the metal analysis (Ag, Cd, Co, Cu, Fe, Mo, Ni, Pb, and V) was verified using the following certified coastal water reference materials for trace metals: CASS- 4 N RC-CNRC. All sampling and analytical operations were carried out according to clean techniques for trace metals. All chemical analyses were performed in duplicate. The results are expressed as ppb ($\mu g \cdot L^{-1}$) for Ag, Cu, Fe, Mo, Ni, and V, and as ppt ($ng \cdot L^{-1}$) for Cd, Co, and Pb.

2.4. Sea urchin embryo-larval assay

2.4.1. Dilution preparation

Prior to the embryogenesis test, the pH of the obtained leachates (pH > 8.5 or pH < 7.0) was adjusted according to CEN/TR 17105:2017 by adding HCL or NaOH 1 N and not exceeding 5% of the total volume of the leachate. This adjustment was carried out so that the leachates could meet the suitable seawater pH (7.0–8.5) conditions for the toxicity test. Five different dilutions were prepared (volume of leachate: volume of seawater): 0:1 (control), 1:4 (25%), 1:2 (50%), 3:4 (75%), and 1:0 (100%).

2.4.2. Biological collection

Biological collection was carried out according to the procedure described by Santos et al. (2023). Briefly, *P. lividus* adults were collected by hand at low tide from a rocky intertidal coast and clean area located close to Santander (NW Spain) and transported in a cooler to the laboratory. Once in the laboratory, gametes were obtained by dissecting mature organisms (three males and three females), and their maturity (sperm mobility and egg sphericity) was examined under an inverted microscope (Motic AE2000) (Rial et al., 2017). The eggs were transferred to a 100 mL graduated cylinder containing clean filtered seawater, and 10 μL of sperm was subsequently added. The mixture was gently shaken to facilitate fertilization. After 30 min, 20-μL aliquots were obtained, and the total number of eggs was counted in a Neubauer counting chamber under an inverted microscope to verify the presence of the fertilization membrane. The fertilization rate (eggs surrounded by a fertilization membrane) was approximately 95%. The acceptability of the samples was fixed at a fertilization rate of 90% (Buttino et al., 2016).

2.4.3. Toxicity test

The embryo-larval development assay was conducted according to the procedure described by Garmendia et al. (2009). Polypropylene vials (20 mL) were filled with different dilutions (5 replicates per dilution) of each leachate. Approximately 500 fertilized eggs were transferred to each vial and incubated for 48 h at 20 °C in the dark. After the incubation period, the larvae in each vial were preserved in 1 mL of 40% formaldehyde and were then observed under an inverted microscope (Motic AE2000).

The percentage of abnormally developed pluteus larvae, which did not have four well-developed arms, per 100 organisms was recorded in each replicate (Khosrovyan et al., 2013). A control treatment (only with clean filtered seawater) was employed to ensure the acceptability of the results obtained, and was fixed when the percentage of normal development was higher than 90%. Two toxicity levels were established: level 0 (normal larvae), four well-developed legs; level 1 (abnormal larvae), four legs not developed. Fig. 2 shows the classification of toxicity levels proposed to assess raw material precursors and AABs.

2.5. Statistical analysis

The data obtained from the metal analysis in leachates and toxicity tests were expressed as the mean ± standard deviation and were calculated using Microsoft Office Excel 2019. The data obtained from

Table 3
Leaching concentration values (ppb and ppt) using seawater as extracting agent (EN 12457–2) for the raw materials (MK: Metakaolin; PV: PAVAL®; WBA: Weathered Bottom Ash; CSP: Glass Recycling Waste), and the Alkali Activated Binders (AA-MK, AA-WBA/PV and AA- CSP/PV).

Sample	ppb (μg·L^{-1})						ppt (ng·L^{-1})				(mS/cm)
	Ag	V	Cu	Fe	Mo	Ni	Pb	Cd	Co	pH	Conductivity
Control	0.91	1.05	0.65	1.37	8.16	0.59	0.1613	0.0490	0.0051	7.88	44.9
MK	6.73	156.37	0.87	2.06	24.11	172.07	2.3486	0.4500	2.1447	6.79	44.8
PV	51.20	4.95	11.86	5.55	548.33	3.01	0.1980	0.5017	0.4855	8.26	42.6
WBA	40.81	20.07	22.56	3.01	16.49	1.26	0.1730	1.2113	0.4758	8.88	45.2
CSP	1.67	1.19	2.25	1.70	9.94	1.10	0.8380	0.1494	0.3292	7.97	44.9
AA-MK	0.34	866.12	0.43	1.13	14.23	0.87	0.1311	0.2276	0.0318	9.13	45.3
AA-WBA/PV	3.28	5.70	6.59	1.46	59.84	1.39	0.0575	0.0520	0.0434	9.91	46.2
AA-CSP/PV	1.76	121.72	9.66	0.14	16.27	1.81	0.3372	0.0306	0.7199	9.80	48.6

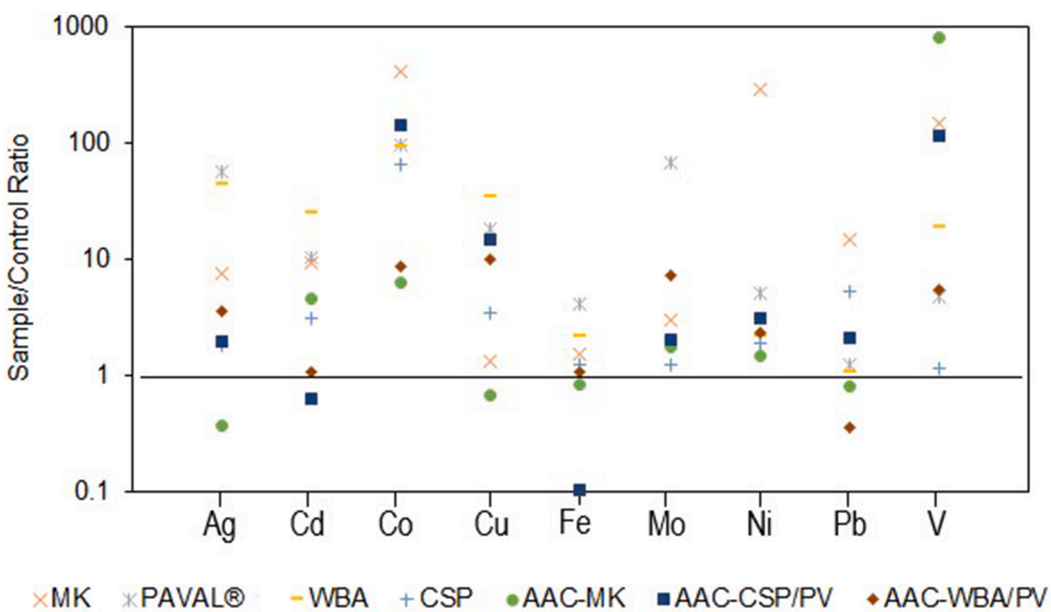

Fig. 3. Chemical analysis results expressed as the relation between the leaching concentration values according to EN 12457–2 in the samples (MK: Metakaolin; PV: PAVAL®; WBA: Weathered Bottom Ash; CSP: Glass Recycling Waste; AA-MK; AA-WBA/PV and AA-CSP/PV) and the control (clean filtered seawater).

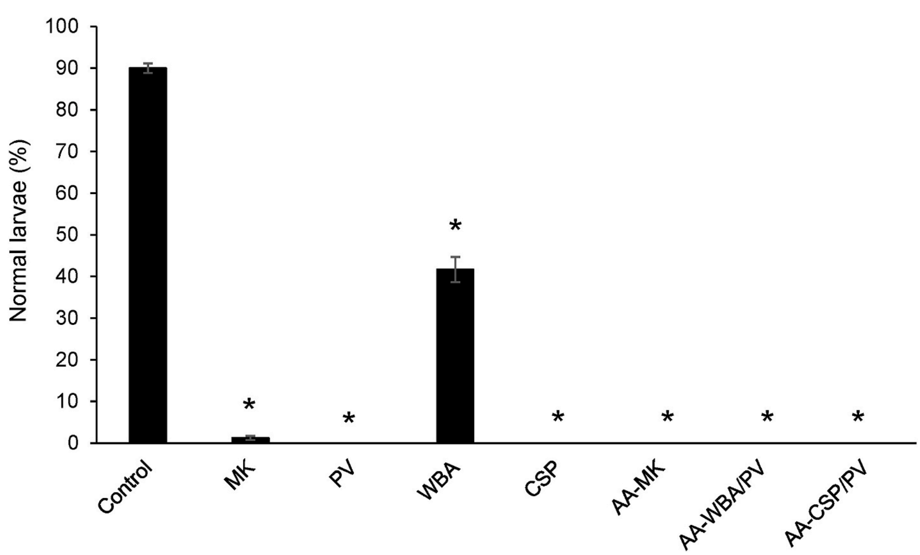

Fig. 4. Percentage of *P. lividus* normal embryos incubated at the different leachates, at 100% of concentration (MK: Metakaolin; PV: PAVAL®; WBA: Weathered Bottom Ash; CSP: Glass Recycling Waste; and the binders, AA-MK; AA-WBA/PV and AA-CSP/PV). The larvae with four legs well developed were considered normal. Bars represent mean ± the standard deviation. Asterisks refer to significant differences to the control treatment (p ≤ 0.05*).

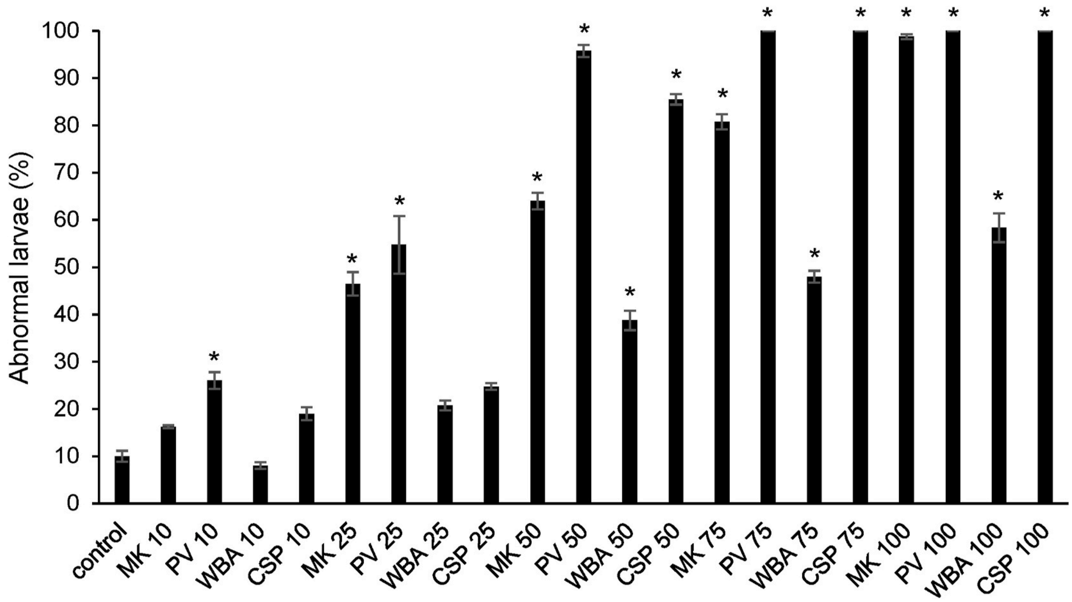

Fig. 5. Percentage of development of *P. lividus* abnormal embryos exposed to different dilutions of raw materials leachates (MK: Metakaolin; PV: PAVAL®; WBA: Weathered Bottom Ash; CSP: Glass Recycling Waste). Bars represent mean ± the standard deviation. Asterisks refer to significant differences to the control treatment ($p \leq 0.05$ *).

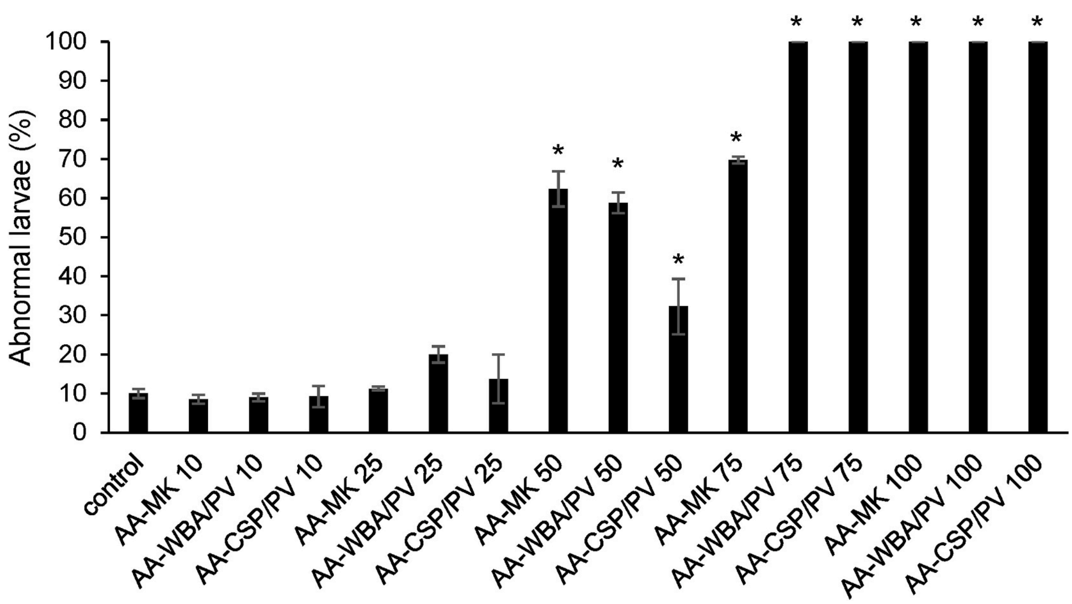

Fig. 6. Percentage of development of *P. lividus* abnormal embryos exposed to different dilutions of Alkali Activated Binders leachates (AA-MK; AA-WBA/PV and AA-CSP/PV). Bars represent mean ± the standard deviation. Asterisks refer to significant differences to the control treatment ($p \leq 0.05$*).

the embryogenesis test were tested for normality and homogeneity using the Shapiro–Wilk and Levene's tests. One-way analysis of variance (ANOVA) with HSD Tukey's post hoc test was performed to test for significant differences ($\alpha = 0.05$) in larval development among the different leachates obtained from the raw material precursors (MK, PV, WBA, and CSP), AABs (AA-MK, AA-WBA/PV, and AA-CSP/PV), and the control treatment (only clean filtered seawater; control). When normality and homogeneity assumptions to satisfy ANOVA requirements were not met, the data were logarithm-transformed (ln +1). The parameter used to express the bioassay results, half maximal effective concentration (EC_{50}), was defined as the leachate concentration that induced negative effects on 50% of sea urchin larvae. This parameter was calculated using dose-response curves that were best adjusted by conducting a non-linear regression analysis using the MOSAIC web interface for statistical analyses in ecotoxicology (Charles et al., 2018). The calculations within MOSAIC were based on the R package (Delignette- Muller et al., 2016). Spearman's rank correlation analyses were used to determine the significant relationship between leachate contaminant content and toxicity on the development of sea urchin larvae. Statistical analyses were performed using the statistical software packages SPSS 21 and GraphPad InStat version 3.00 for Windows.

3. Results and discussion

3.1. Leaching behavior

The leaching behaviors of the precursors (MK, PV, WBA, and CSP)

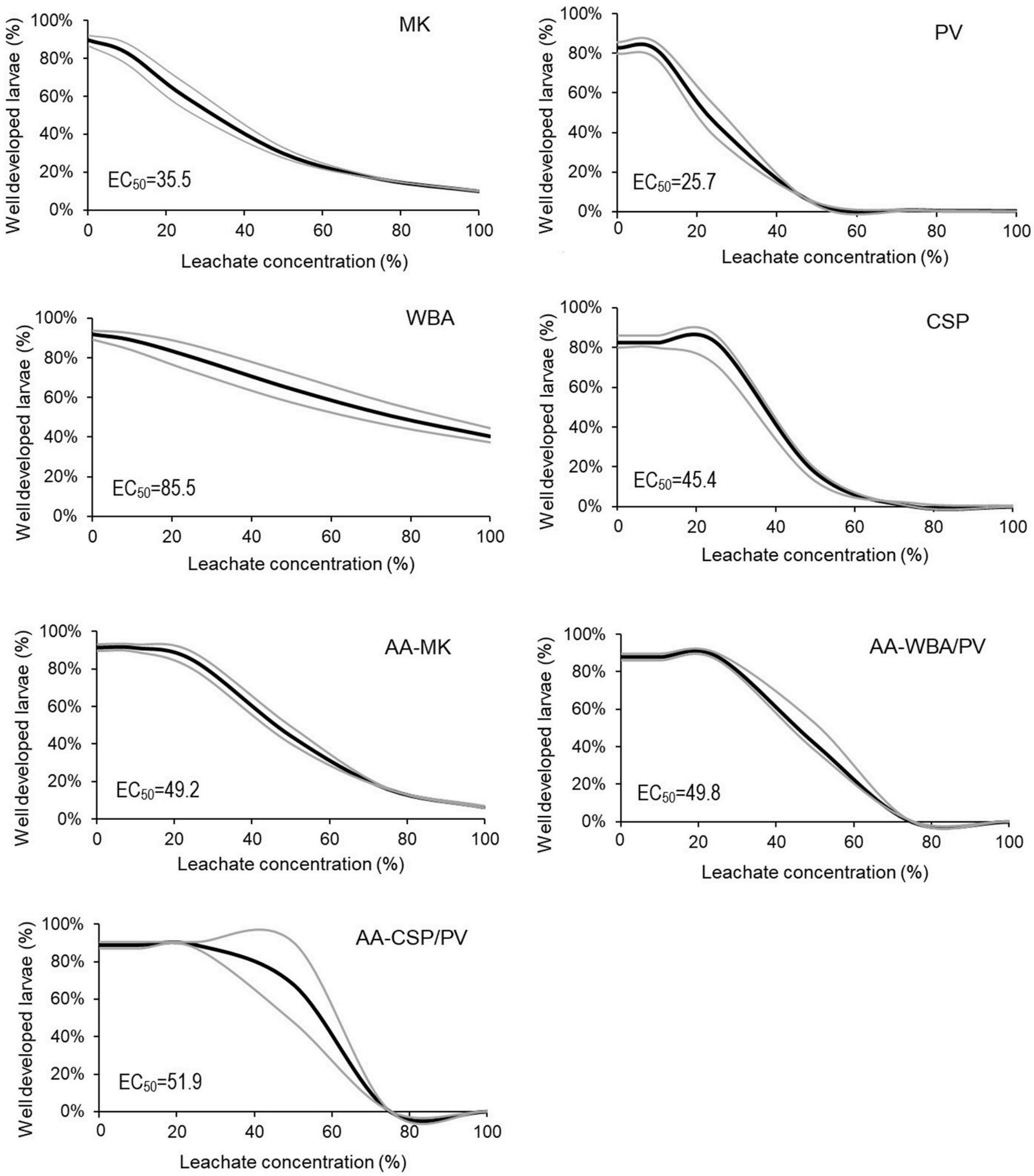

Fig. 7. Early development dose-response curves of *P. lividus* normal embryos exposed to the different leachates (MK: Metakaolin; PV: PAVAL®; WBA: Weathered Bottom Ash; CSP: Glass Recycling Waste; and the binders, AA-MK; AA-WBA/PV and AA-CSP/PV) in an embryogenesis test. Bars represent mean ± the standard deviation. Asterisks refer to significant differences to the control treatment ($p \leq 0.05$*).

and binders (AA-MK, AA-WBA/PV, and AA-CSP/PV) were evaluated based on the contaminant mobility criteria. According to the results of the leaching test, it is important to note the complexity of the matrices because of the heterogeneity of the waste materials/industrial by-products used as well as the pH values that reached the leachates of the alkali-activated binders. In addition, the leaching liquid used, seawater, provides high salinity, thereby increasing the difficulty of chemical analysis. Table 3 shows the metal content and pH values of the leachates obtained according to the leaching test procedure EN 12457–2 proposed by European regulations (CEN, 2003), but conducted with seawater as a leaching agent to simulate the potential behavior of different materials in the marine environment. The results, expressed as the ratio between the leaching concentration values and the control (clean filtered seawater), are shown in Fig. 3. In terms of the raw materials as precursors, most mobility results were at a ratio of approximately less than 10, except for the values of Co, Ni, and V for the MK sample and Mo for the PV sample, which had ratios greater than 100. This behavior could be due to the different pH values of leachates (6.79–8.88) and in seawater (7.88) as well as the presence of other elements, such as Fe, that act as complexing agents (Coronado et al., 2015).

The mobilization of all elements was considerably reduced when comparing the leaching behavior of the AABs with the corresponding precursors used. These values were very close to the values of the background levels of seawater, except in the case of V. In terms of the AABs, AA-CSP/PV exhibited the best leaching behavior for Cd and V, despite being synthesized only with industrial byproducts. In contrast, the reference binder AA-MK, which uses a virgin raw material (calcined kaolin), exhibited the worst results. Therefore, it could be assumed that the large amounts of vanadium mobilized in AA-MK leachates could come from the MK load, as with other clays (Cifrian et al., 2021; Maldonado-Alameda et al., 2021), as well as the presence of molybdenum from the PV load or copper from the PV and WBA, in other alternative material leachates.

In this study, the potential hazard of materials was evident when the obtained metal mobility results were compared with those of the control

Table 4
Effective Concentrations NOEC (Non-Effect Concentration, EC_{10}), EC_{50} and EC_{80}, for different leachates (MK: metakaolin, PV: PAVAL®, WBA: Weathered Bottom Ash, CSP: Glass Recycling Waste, and the binders, AA-MK, AA-WBA/PV and AA-CSP/PV) in an embryogenesis test. Considering as endpoint the lack of four well-formed legs. EC_x values are expressed as leachate dilution percentage and 95% confidence interval.

	MK	PV	WBA	CSP
NOEC	11.7 (9.25–14.1)	15.9 (13.7–17.8)	21.2 (16.7–26.2)	38.6 (24.5–47.8)
EC_{50}	35.5 (32.3–38.5)	25.7 (24.1–27.2)	85.5 (78.2–94.4)	45.4 (36.7–49.2)
EC_{80}	71.1 (65.7–77.1)	34.8 (33.0–36.8)	210 (173–256)	49.9 (46.8–50.9)
	AA-MK	**AA-WBA/PV**	**AA-CSP/PV**	
NOEC	27.0 (24.4–29.7)	45.7 (41.7–48.7)	48.5 (46.7–49.5)	
EC_{50}	49.2 (46.9–51.5)	49.8 (49.1–50.2)	51.9 (50.6–53.8)	
EC_{80}	71.7 (68.6–75.3)	52.5 (50.7–55.2)	54.2 (51.4–58.4)	

(clean filtered seawater). Despite these results, there is a lack of a regulatory or reference framework to compare this type of material, both for freshwater and seawater.

3.2. Sea urchin embryo-larval development test

During the embryonic development of the sea urchins, embryos were exposed to different leachate dilutions from raw materials (MK, PV, WBA, and CSP) and AABs (AA-MK, AA-WBA/PV, and AA-CSP/PV). After 48 h of exposure, a wide range of developmental and morphological effects were recorded, such as the number of larvae that had developed four arms, those on which arms had begun to grow, those that had not started development, and the ovules that appeared destroyed.

In terms of the statistical analysis of the data, the results were expressed according to two different toxicity levels. The first-level groups included pluteus larvae with four well-developed arms. The second-level groups had no recognizable larval structure or did not pass the first stages of growth.

Fig. 4 shows the percentage of normal pluteus larvae observed for leachates obtained from raw material precursors and AABs. The average number of normally developed larvae in the control treatment was >90%, thereby validating the acceptability of the test (Volpi Ghirardini et al., 2005). High toxic effects on sea urchin larval development were reported after exposure to all undiluted leachates studied, with percentages of normal pluteus lower than 1.5%, except for WBA (41.6% of normal larvae). In addition, the toxicity exhibited by all the leachates was statistically different from that of the control treatment ($p < 0.05$), indicated by the asterisk (*). Except for WBA and MK, the remaining leachates blocked early embryonic development, and the first larval stage of the sea urchin, known as the pluteus stage, was not reached.

Figs. 5 and 6 show the percentages of abnormal development in embryos exposed to different dilutions of raw materials and AAB leachates, respectively. Specifically, after 50% of the leaching concentration, there were significant differences in all raw materials with the reference with a level of significance of 95% ($p \leq 0.05$*). In the case of MK, this notable difference appeared from 25% dissolution, and for PV, from a concentration of 10% leachate, indicating its more significant toxicological potential. Finally, the AAB results indicate the existence of significant differences between the control and the leachates from a concentration of 50% of leachate for all cases at a level of significance of 95% ($p \leq 0.05$*).

The best fit of the dose-response curves for all the leachates was calculated to obtain the EC values for each raw material and AABs (Fig. 7). Table 4 shows the non-effect concentration (NOEC), EC_{50}, and EC_{80} values. For the value of NOEC, EC_{10} was used (Oliva et al., 2021). These results show how the leachates from MK, PV, and WBA are the ones that need less concentration to start affecting the embryonic development of the sea urchins. In contrast, CSP requires a higher concentration to begin showing its effect on embryonic development. In the case of PV, this trend was maintained, being, for all cases, the one that required a minor leachate concentration to affect embryonic development. WBA showed the opposite trend, requiring higher concentrations to affect the normal development of *P. lividus* larval specimens. In order to affect 70% of the exposed embryos a leachate concentration of 100%, $EC_{80} = 210$ was required. MK followed the trend of the WBA but was much softer, requiring 71.1% of leachate concentration to affect 80% of the sea urchin larvae. Finally, although CSP required high concentrations to begin to affect the sample (NOEC = 38.6%), a concentration of 49.9% was required to affect 80% of the urchin embryos.

In the case of AABs, the reference binder (AA-MK) is the one that needs the lowest concentration to start affecting the embryos (NOEC of 27%). However, it was the one that required the highest concentration to affect 80% of the of sea urchin larvae. The other binders (AA-WBA/PV and AA-CSP/PV) exhibited similar trends. Both required a high leachate concentration to start affecting sea urchins with NOEC of 45.7% and 48.5%, respectively, but slightly higher concentrations to fully affect embryonic development with EC_{80} of 52.5% and 54.2%, respectively.

In terms of the EC_{50} values, the raw materials exhibited significant differences between them. PV showed the highest toxic impact on larval development (25.7%), while metakaolin (MK) and glass recycling waste (CSP) showed minor impacts (35.5% and 45.4%, respectively). The highest EC_{50} value was reported for weathered bottom ash (WBA), indicating lower toxicity to sea urchin larval development. In the case of AABs, the EC_{50} values were approximately 50% in AA-MK, AA-WBA/PV, and AA-CSP/PV. The EC_{50} values of alkali-activated binders were higher than those of the raw material precursors (except for WBA), which means that they could have a minor impact on the marine environment based on the toxicity produced by sea urchin larvae. These results agree with the reduced mobility of contaminants recorded in the products leachates.

These toxicity values obtained are in line with a previous study of some authors of this work (Santos et al., 2023), in which EC_{50} values for coal fly ash based on artificial reef materials were obtained using the same bioassay and endpoint, with geopolymer mortars showing a greater toxic effect than cement mortars (11.8% and 52.3%, respectively). It has been previously reported in the literature that the embryonic development of sea urchins (teratogenic effects) is affected by the presence of some metals, such as Ni or Cu (Bonaventura et al., 2018; Manzo et al., 2010; Morroni et al., 2018; Novelli et al., 2003) or their combined toxicity (Fernandez and Beiras, 2001).

It is important to highlight that, the abnormalities found in the case of the raw materials, MK, PV, and WBA, are mainly in the developing leg stages (i.e., lack of skeleton, four legs not developed, and too short legs).

Table 5
Spearman correlation coefficients relating metal content in leachates obtained from raw materials precursors and alkali-activated binders and percentage of abnormal larvae.

	Ag	Cd	Co	Cu	Fe	Mo	Ni	Pb	V
Larval toxicity	0.047	−0.041	0.284	0.105	−0.099	0.382[a]	0.372[a]	0.081	0.333[a]

[a] Significant correlations at $p \leq 0.05$ are marked with one asterisk.

In the literature similar effects have been reported as a consequence of the contents of vanadium (Fichet and Miramand, 1998), nickel, lead (Fernández and Beiras, 2001), zinc (Camacho et al., 2018), and copper (Kobayashi and Okamura, 2004) with reference toxic samples or in more complex samples with different concentrations of glyphosate-based products (Asnicar et al., 2020).

On the other hand, the results showed that CSP and AABs leachates at high dilution (over 75%) tended not only to affect the development of organisms, but also inhibit it in the early stages. This response was similar to that reported in the literature associated with the presence of high Ni concentrations (Bonaventura et al., 2018) or when testing different microplastic sets (Trifuoggi et al., 2019) and plastic toys (Oliviero et al., 2019).

3.3. Linking metal mobility and biological effects

Spearman's correlation links metal leaching from raw materials and AABs with abnormal larval development observed in *P. lividus* (Table 5). The correlation analysis identified highly significant correlations ($p < 0.05$) between the presence of Ni and Mo in leachates and larval toxicity and a certain correlation with the presence of V. However, the correlation analysis did not indicate significant relationships between the concentrations of the remaining elements analyzed in leachates and abnormal larval development.

The results suggested that the lowest EC_{50} values calculated for MK (35.5%) and PAVAL® (25.7%) were mediated by the mobility of Ni (172 μg·L-1) and Mo (548 μg·L-1), respectively. However, the EC_{50} determined for PV, an industrial by-product, is in contrast with the waste acceptance criteria based only on the chemical analysis of leachates, which classified PV as a non-hazardous material according to the European Waste Landfill Directive (EU, 2003). This result highlights the importance of assessing the potential ecotoxicological impact of construction materials.

On the other hand, in the literature, Volpi Ghirardini et al. (2005) showed the first malformed larvae in *P. lividus* for Cu solutions at 50 μg·L-1, while, Dermeche et al. (2012) reported LC_{50} (median lethal concentration) toxicity of Cu (158.48 μg·L-1), Cd (61.65 μg·L-1), and Pb (446.68 μg·L-1) solutions. These reported values are higher than the concentration values discussed in the present study (Table 3).

In relation to Ni toxicity in seawater, several studies have been published using sea urchin *Strongylocentrotus purpuratus* embryos. Ryu et al. (2012) observed malformations >90% in the sea urchin larvae exposed to Ni concentrations of 100 μg L^{-1}. Blewett et al. (2018, 2016) showed EC_{50} values of 207 μg L^{-1} (167–247 μg L^{-1}) of Ni. Recently, the relation between the complexation of Ni and its toxicity has been characterized (Sherman et al., 2021). These results agree with the similar Ni concentration found in the raw material MK (172 μg L^{-1}), which was the second most toxic leachate in terms of EC_{50} in the present study.

Finally, from a technical point of view, it can be concluded that the results obtained from bioassays show great robustness, presenting significant differences (95%) between the control and the leachates of precursors and binders and with very similar results from the replicates. As future research, to achieve greater sensitivity to potential effects on the environment, the registration of more levels of toxicity (endpoints) will be proposed, which reflect more teratogenic effects in the development of embryos, and consequently much more reliable statistical results will be obtained. Furthermore, this bioassay will be applied to other alkaline activated matrices, in order to evaluate its implementation in a legal framework.

4. Conclusion

The present study analyzed the ecotoxicological potential of raw material precursors (MK, PV, WBA, and CSP) and their corresponding three alkali-activated binders (AABs) through leaching and toxicity in the larval development of the sea urchin *P. lividus*.

The mobilization of all contaminants in the raw materials is considerably reduced by alkaline activation, except for vanadium. The loads acquired of Mo, Cu, and V can be traced from the raw materials to AABs.

The sea urchin embryogenesis bioassay exhibited significant differences between the control and all the leachates at a level of significance of 95%. The values of EC_{50} (the leachate concentration that induces negative effects on 50% of sea urchin larvae) for alkali-activated binders (AA-MK, AA-WBA/PV, and AA-CSP/PV) were approximately 50%. These values were higher than in raw material precursors (except for WBA, 85%), which means that they could have a minor impact based on the toxicity produced on sea urchin larvae. These results agree with the reduced mobility of contaminants recorded in the products leachates. The correlation between the mobility of pollutants and toxicity allows the identification of molybdenum and nickel as the most determining elements, showing significant correlation coefficients and a certain correlation with the presence of vanadium.

The results suggest that the sea urchin embryo development test, owing to its great sensitivity and ecological relevance, is an excellent candidate as aquatic toxicity test for inclusion in a battery of bioassays designed to evaluate the potential risks associated with construction products in the marine environment. In this sense, our findings provide information useful to consider the use of marine aquatic toxicity tests to classify construction materials according to their hazard in a legal framework. Further research should be focus on the identification of potential ecotoxicity tests which allow us the development and optimization of a complete and innovative battery of bioassays to advance in the knowledge of the toxic effects of construction materials on marine environment and their subsequently implementation in an environmental policy.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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