Behavior of Concrete and Mortar in Response to the Inclusion of Toxic *Jatropha* Seed Cake

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As the world's leading civil construction materials, concrete and mortar are the focus of ongoing studies aimed at improving their properties. These materials are highly versatile; hence, some of their aspects, such as their interaction with toxic materials, should be examined in greater depth. An investigation was therefore undertaken to ascertain how these products react to phorbol ester (PE), a toxin found in *Jatropha* seed cake (JSC). The mechanical behavior of mortar and concrete containing JSC waste from the manufacture of biofuel was examined based on the analysis of axial compressive strength. The interaction between mortar and PE molecules was examined by means of high performance liquid chromatography. A study of the mechanical behavior of the materials indicated that the inclusion of JSC greatly reduced their mechanical properties, and that this inclusion had a stronger impact on mortar than on concrete, while liquid chromatography showed that the toxic material inserted into the mortar remained inert, indicating the promising potential of this material to store toxic products.

Keywords: Concrete; Sustainability; Toxic material recycling

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INTRODUCTION

Civil construction is one of the pillars of Brazil's economy, and the main product used in this sector is concrete (Iqbal and Quiaswari 2012), which is artificial rock composed of binders, fine and coarse aggregates, additives, and water. Concrete is consolidated in infrastructures in its hardened form; hence, its physicochemical properties are the object of in-depth studies. In engineering works, the most commonly investigated characteristics of hardened concrete are its axial compressive and flexural strengths, which are notable properties of columns and beams.

In response to growing concerns about environmental issues, construction-related research has begun to use alternative materials in concrete production. This research also involves analyzing the material's behavior before the inclusion of toxic wastes, as concrete tends to stabilize toxic material (Souza *et al*. 2010).

Jatropha seed cake (JSC) is the solid waste generated by crushing the seeds of *Jatropha curcas* L. (common name: physic nut or Barbados nut) to extract their oil. Besides sugarcane, which is currently used in the production of biofuels, *Jatropha* oil shows a promising potential as a biofuel in Brazil. Other inedible vegetable oils are gaining attention for their potential to replace fossil fuels (Lee *et al.* 2011).

Although JSC contains various antinutritional chemical compounds such as phytic acid and trypsin inhibitors, Devappa *et al*. (2011) stated that phorbol ester (PE) is by far the most hazardous component due to its carcinogenicity. These authors also studied the composition of PE in the various parts of *Jatropha curcas* seed.

The purpose of this study was to analyze the behavior of mortars and concretes containing toxic elements, particularly their mechanical strength and their ability to retain such toxins. As PE is a proven harmful organic compound, JSC in different proportions was inserted as an aggregate into concrete and mortar test specimens, which were then cast. The material behavior was analyzed based on axial compression tests and high performance liquid chromatography (HPLC).

EXPERIMENTAL

Materials

The mortars were cast using cement as a binder and sand and JSC as aggregates. Two types of coarse aggregate, CAφ12.5 and CAφ19.0, were added to the concrete mix for casting. The sand and JSC were tested to determine their specific gravity and water absorption, as specified by ASTM C128-15 (2015), while the diameter of the aggregates was determined according to ASTM D422-63 (2007). JSC (Fig. 1) was added to the mixture in the same percentage as sand. Table 1 describes the physical properties of the aggregates.

Fig. 1. *Jatropha* seed cake used in the experiments

The CAφ12.5 and CAφ19.0 were tested to determine their specific gravity and absorption according to ASTM C127 (2015). The aggregate diameter was ascertained based on ASTM C33/C33M (2016).

Table 1. Aggregate Properties

Aggregates	Specific Gravity (g/cm³)	Water Absorption (%)	Diameter (mm)
Cement	3.07	-	-
Sand	2.61	0.34	1.18
JSC	1.25	0.38	2.36
CS0	2.89	1.43	12.50
CS1	2.89	1.24	19.00

The mixture was prepared using a cement-to-dry sand ratio of 1:2.06. Six different mortar compositions were prepared with varying amounts of sand and JSC, as listed in Table 2. MREF is the mortar of reference, *i.e.*, no addition of JSC, while MJSC2, MJSC4,

MJSC6, MJSC8, and MJSC10 are mortar compositions containing 2, 4, 6, 8, and 10% JSC instead of the mass of the sand, respectively. JSC was placed for replacement of sand due to the maximum diameter thereof, which would conform to the diameter of a sand (Table 1). That is, the JSC is not increased in concrete, but replaced by sand (by mass).

Table 2. Proportion of Aggregates in Mortar Compositions

Aggregates	Proportions					
	MREF	MJSC2	MJSC 4	MJSC 6	MJSC 8	MJSC 10
Cement	1.00	1.00	1.00	1.00	1.00	1.00
Sand	2.06	2.02	1.98	1.94	1.90	1.88
JSC	0.00	0.04	0.08	0.12	0.16	0.20
Water	0.53	0.46	0.48	0.47	0.51	0.53

Table 3 describes the variations in the amount of aggregates in the concrete. The abbreviation CR corresponds to the concrete of reference, *i.e*., without the addition of JSC, while CJSC2 and CJSC4 are the concrete compositions containing 2 and 4 wt.% of JSC, respectively, in place of the same amounts of sand.

Table 3. Proportion of Aggregates in Concrete Compositions

Aggregates	Mix		
	CR	CJSC2	CJSC4
Cement	1. 00	1. 00	1. 00
Sand	2. 06	2. 02	1. 98
CAϕ12.5	2. 06	2. 06	2. 06
CAϕ19.0	0. 88	0. 88	0. 88
JSC	0. 00	0. 04	0. 08
Water	0. 53	0. 46	0. 48

Preparation of test specimens

Four 5 × 10 cm cylindrical test specimens of each of the six mortar compositions, MREF, MJSC2, MJSC4, MJSC6, MJSC8, and MJSC10, were prepared for mechanical testing. The mortars were cast as specified by the ASTM C1329 standard (2016). In addition, three 10 × 20 cm cylindrical test specimens of the concrete compositions CR, CJSC2, and CJSC4 were cast for axial compressive strength testing. The concrete was cast as specified by ASTM C192/C192M (2016). The mortar and concrete test specimens were cured in a humidity chamber at a temperature of 25 ºC and air humidity of 95%.

Compressive strength test of cylindrical concrete specimens

The samples were cured in the humidity chamber until they ruptured. Both the mortar and concrete specimens ruptured at 7, 28, and 90 days. Testing of the mortars was performed on three samples of each composition and age, according to ASTM C348 (2014), while all the concrete test specimens were tested according to ASTM C39/C39M (2017).

Preparation of specimens for HPLC testing

Figure 2 illustrates the mortar test specimens prepared for HPLC testing, which came from the same lot as those cast for mechanical testing. Samples of pure JSC and of mortar compositions MJSC2 (2%) MJSC4 (4%), MJSC6 (6%), MJSC8 (8%), and MJSC10

(10%) were selected for this test and were divided into four ages, namely, 7, 28, 90, and 120 days. Each of these samples was immersed in 500 mL of water inside a plastic container, as illustrated in Fig. 3.

Fig. 2. Mortar test specimens

Fig. 3. Mortar samples immersed in water

After the samples completed their respective periods of immersion, they were removed from their containers, and the water was stirred to homogenize it. Two aliquots of 10 mL of water were then immediately removed from each container for HPLC.

Chromatographic process

Phorbol 12-myristate 13-acetate (PMA) (99% purity) was purchased from Sigma Aldrich (Saint Louis, USA). The solvents used in this study, acetone (PA grade), acetonitrile (ACN), dichloromethane (DCM), methanol (MeOH), and trifluoroacetic acid (TFA) were of HPLC grade (99.95%; JT-Baker, México).

The PE-rich fraction was separated using a Shim-pack C_{18} HPLC analytical column (250 mm x 4.6 mm ID, 5.0 particles; (Kyoto, Japan) and two LC-20AT and LC-20AD pumps. The chromatographic peaks were detected using a Shimadzu SPD-M20A detector (Kyoto, Japan) operating in the range of 200 to 800 nm. The chromatographic peaks were separated by means of an elution gradient using Milli-Q water (solvent A) acidified with 0.01% TFA and ACN (solvent B) at a ratio of 95:5 (v/v) for 3 min, followed by 50:50 (v/v) for 4 min, a gradual increase of 5:95 (v/v) for 5 min, and completion of 18 min. The volumetric flow rate was 1.0 mL/min^{-1} at room temperature.

Extraction of phorbol ester (PE) extracts by dispersive liquid-liquid microextraction

The water samples from the immersion of samples were subjected to dispersive liquid-liquid microextraction (DLLME) as described by Martins *et al*. (2012). The DLLME was performed in the following steps: 1) 5.0 mL of water sample was placed in a conical test tube (15.0 mL) and 0.25 mg of NaCl was added to it, after which it was vortexed for 5 min; 2) Acidification with HCl (0.01 M) to pH ~3.0; 3) Vortex for 2 min; 4) Addition of 500 μL of acetone (dispersing agent) and 500 μL of DCM (extractive agent); 5) Vortex for 5 min; 6) Separation of the organic phase from the aqueous phase using a microsyringe (100 μL); 7) Drying of the eluate at 40 ºC under a nitrogen gas flow; 8) Resuspension of the sample with 500 μL of ACN, and chromatographic injection (in triplicate).

Figures of merit (calibration model)

Net analyte signal (NAS) plays an important role in the calculation of figures of merit for characterizing a calibration model (Faber and Kowalsky 1997). Among the most important figures of merit, sensitivity, selectivity, and the limits of detection (LOD) and quantification (LOQ) pertain to the concept of NAS. This concept plays an important role in the calculation of figures of merit to characterize a calibration model (Roque *et al*. 2017). The parameters for validation of the method were described by Cass *et al.* (2011). *Sensitivity* is the method's ability to distinguish two similar concentrations with a given confidence level. *Selectivity* is the method's ability to separate components of the sample that will be visible from the compound of interest. *Limit of Detection* (LOD) represents the lowest concentration of the test substance that can be measured using a given experimental procedure. *Limit of Quantification* (LOQ) is expressed as a concentration, and the precision and accuracy must also be recorded.

RESULTS AND DISCUSSION

Mechanical Tests on Mortars and Concretes

Table 4 describes the axial compressive strength of mortar containing six different proportions of JSC. The axial compressive strength of the mortar samples diminished in proportion to the increasing amounts of JSC in place of sand, as shown in Table 4. The axial compressive strength of mortar compositions containing 2, 4, 6, 8, and 10% of JSC in place of sand decreased at 7 days by 55, 88, 98, 90, and 99%, respectively, at 28 days by 54, 86, 88, 96, and 98%, respectively, and at 90 days by 44, 75, 91, 95, and 97%, respectively. This decrease in axial compressive strength was attributed to the addition of organic material to the mortar, which presumably increased the number of voids and diminished the uniformity of the cementitious bonds, causing structural failures in the test specimens and consequent decreases in their mechanical strength. In addition, small strengths for JSC mortars may have occurred due to the fact that they do not have crushed stone, which increases the strength of the concrete and is added by JSC, which decreases the strength of the concrete. However, although there is a 54% reduction in mortar strength, at 28 days with 2% JSC, compared to the reference mortar of the same age, it can still be used in non-load bearing walls. For larger amounts of JSC in the mortar, there was a devastatingly negative effect on the strength. The reference mortar obtained compressive strength values for load-bearing walls (ASTM C270-14a).

However, upon adding the coarse aggregate to the concrete mixture, the decrease in axial compressive strength was less pronounced, as shown in Table 4. The axial compressive strength of concrete compositions containing 2 and 4% of JSC in place of

sand decreased at 7 days by 22 and 27%, respectively, at 28 days by 10 and 34%, respectively, and at 90 days by 10 and 21%, respectively. Thus, the concrete was less sensitive to the addition of 2 and 4% of JSC than the mortar.

According to the values of compressive strength, the concrete can be indicated for structural masonry, subfloor, sidewalk and slab, provided that no other floor is built on it.

Table 4. Axial Compressive Strength of the Mortar Reference Sample and Mortar Samples Containing Different Amounts of JSC in Place of Sand in MPa

			JSC (%)					
Materials	Age (days)	Samples	0	2	4	6	8	10
Mortar	7	S1	3.55	1.96	0.28	0.06	0.05	0.08
		S2	4.85	2.37	0.62	0.18	0.06	0.08
		S3	5.21	1.32	0.51	0.06	0.06	0.06
		Average	4.67	2.08	0.55	0.10	0.06	0.07
		SD*	0.757	0.579	0.209	0.066	0.007	0.007
	28	S1	5.77	2.90	0.85	1.12	0.25	0.13
		S2	6.63	2.99	0.88	0.47	0.20	0.14
		S3	6.20	2.71	0.83	0.74	0.27	0.19
		Average	6.20	2.87	0.85	0.78	0.24	0.15
		SD*	0.433	0.143	0.025	0.326	0.034	0.034
	90	S1	6.99	4.19	2.28	0.66	0.24	0.37
		S2	7.50	3.91	1.59	0.70	0.19	0.34
		S3	7.61	4.28	1.8	0.65	0.38	0.25
		Average	7.37	4.13	1.89	0.67	0.23	0.37
		SD*	0.332	0.193	0.353	0.027	0.031	0.019
Concrete	7	S1	18.69	14.60	15.34	-	-	-
		S2	15.87	17.86	14.35			
		S3	20.99	12.83	11.52			
		Average	18.73	14.73	13.77			
		SD*	2.139	2.206	1.620			
	28	S1	25.27	21.94	19.13			
		S2	25.56	23.47	16.23			
		S3	26.62	21.96	14.41			
		Average	25.08	22.55	16.67			
		SD*	1.579	0.822	1.949			
	90	S1	24.30	20.54	21.23			
		S2	25.15	27.21	16.03			
		S3	28.77	24.49	21.96			
		Average	26.07	23.56	20.63			
		SD*	2.374	2.931	3.179			

*Standard Deviation

Identification and quantification of PE extraction peaks resulting from JSC

Chromatographic peaks of PE (five) were found between 6.85 and 12.12 min (Fig. 4.) and were monitored at 280 nm. The retention time (tR) (time it takes for a solute to travel through the column) of each of the profiles is also shown. The area of each peak was expressed as equivalents of PMA, which was used as a marker and eluted at 12.12 min.

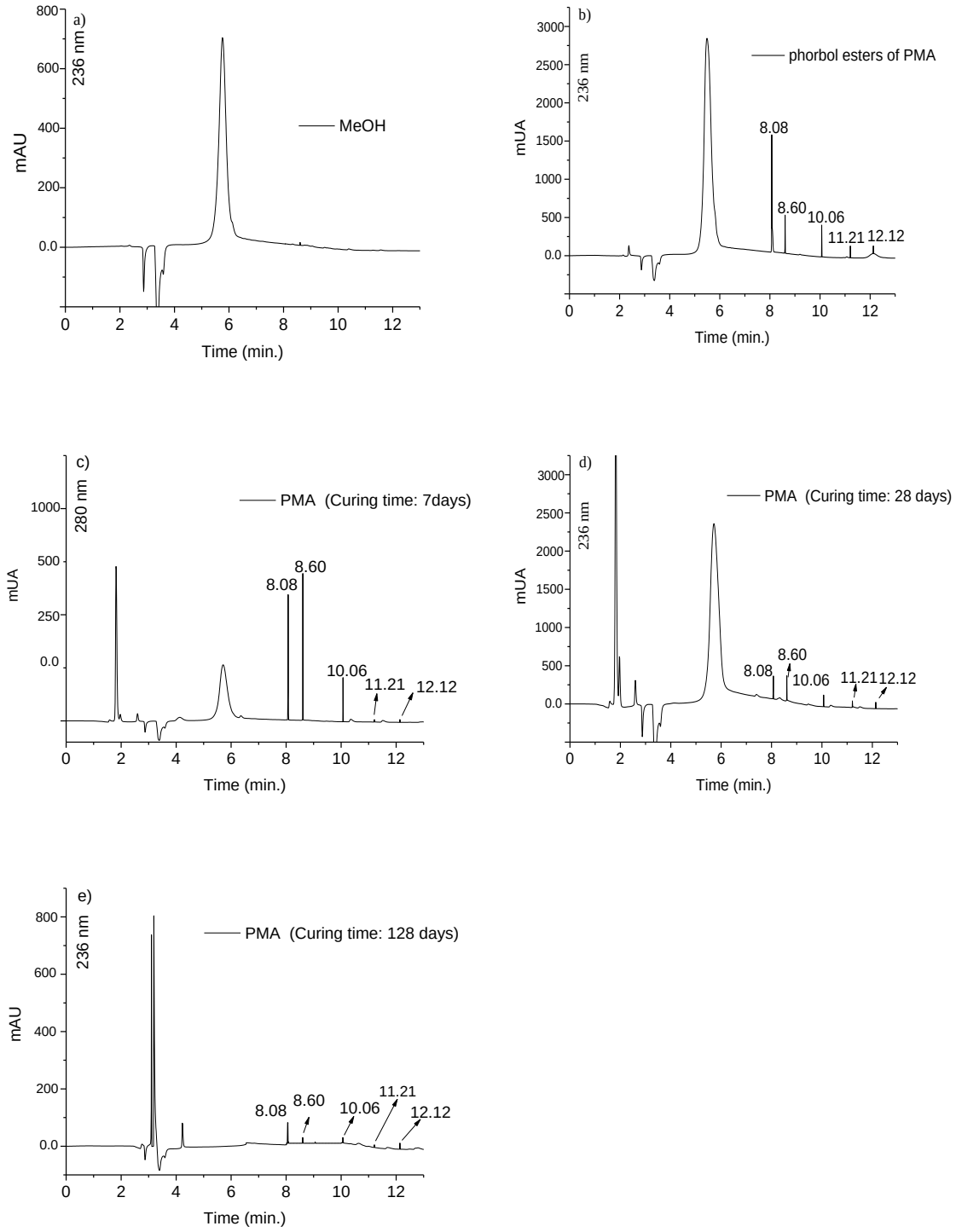

Fig. 4. Chromatogram acting: (a) MeOH, (b) phorbol esters of PMA which are epimers: Tr: 8.08 min; Tr: 8.60 min; Tr: 10.06 min; Tr: 11.21 min and Tr: 12.12 min., (c) curing time 7 days, (d) curing time 28 days and (e) curing time 120 days

The regression equation obtained to quantify the extracted PE was [PE] = 2.754*x* + 3.10, where SA corresponds to the sum of the areas of chromatographic peaks, and [PE] is the concentration of PEs. Linearity, which was expressed by R^2, was equal to 0.9988. This value indicated high linearity and sensitivity of the model within the examined

concentration range (1.55, 3.10, 6.30, 12.50, and 25.00 μg L^{-1}).

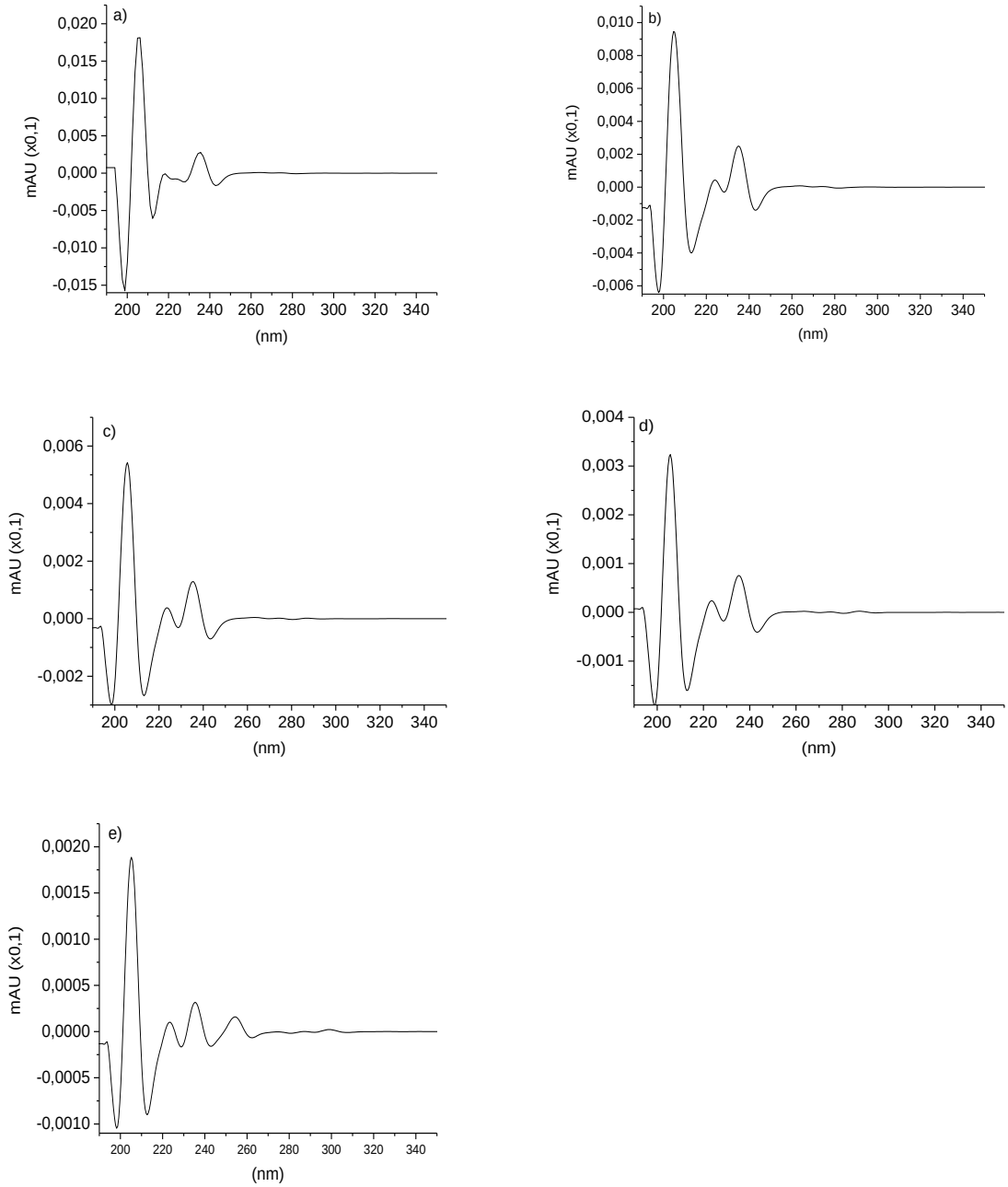

Fig. 5. Spectra of the chromatographic peaks of the PMA. a) Tr: 8.08 min, b) Tr: 8.60 min, c) Tr: 10.06 min, d) Tr: 11.21 min e) Tr: 12.12 min

Precision was evaluated as a measure of the reproducibility of the entire analytical method, and was expressed by the relative standard deviation (RSD) calculated by the proportion of the standard deviation and the mean of the areas of chromatographic peaks of five repetitions. The obtained RSD value of 3.54% was considered appropriate and acceptable, in view of the complexity of the samples. According to the $t_{calculated}$ value (0.679) for PE, there was not a significant difference between the recoveries obtained and the expected value (100%) of the three strengthened samples (1.25, 2.50, and 5.00 μg L^{-1}), as the absolute value of $t_{calculated}$ was lower than the t table value. Hence, the

chromatography quantification method was accurate.

The LOD and LOQ (*i.e.*, the lowest concentration of an analyte in a sample that can be determined with acceptable accuracy and precision) were determined by calculating a signal to noise ratio of 3:1 (LOD) and 10:1 (LOQ) between the standard deviation value in relation to the chromatographic signal of seven injections of MeOH as the blank and the slope of the calibration curve. The LOD and LOQ found here were 0.543 and 1630 μg L^{-1}, respectively.

To confirm the presence of PE, the chromatographic peaks of PMA, relative to JSC, were evaluated in terms of their spectrophotometric absorption profiles (Fig. 5.).

Toxin-mortar interaction at different ages

Table 5 and Fig. 6 describe the PE content (mg L^{-1}) in the test specimens containing JSC at the different water immersion times (days).

Table 5. PE Content (in mg L^{-1}) in Test Specimens Containing JSC Immersed in Water for Different Lengths of Time

Discretization of the Samples	PE mass released into water (mg)					
	7 days	28 days	90 days	120 days	Average	SD*
MSC2	0.28	0.36	0.36	0.12	0.28	0.117
MJSC4	0.34	0.41	0.43	0.42	0.4	0.038
MJSC6	0.38	0.44	0.39	0.15	0.34	0.130
MJSC8	0.43	1.00	0.65	0.43	0.63	0.268
MJSC10	0.87	1.28	0.38	0.39	0.73	0.435
*Standard Deviation						

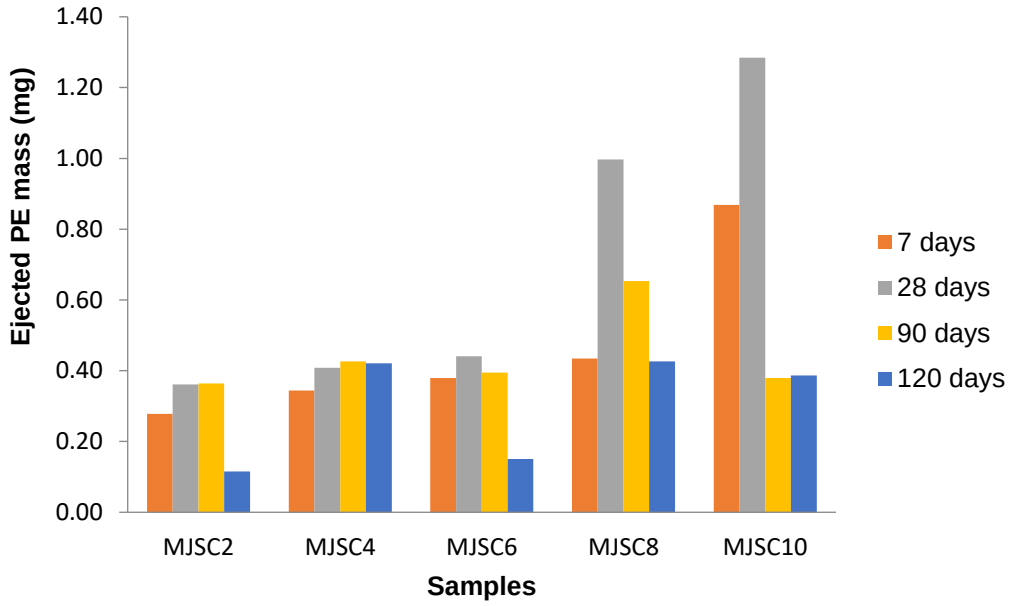

Fig. 6. PE content released by mortars containing JSC

The amount of PE eliminated into the water varied according to the age and the quantity of JSC added as aggregate to the mortar samples. The samples containing 8% and 10% of JSC at the age of 28 days, and those containing 2% and 6% at the age of 120 days were less stable than the other samples. Notwithstanding the discrepancy in the abovementioned specimens, the average amount of PE released by the samples remained

between 0.4 and 0.5 mg.

A comparison of the values of PE mass released and of PE mass retained in the hardened mortar samples enables a broader bias of the process of release of the compound. The values of PE mass in each sample were estimated based on the PE fraction found in the JSC batch assayed by HPCL, which was 1.2746 mg/g. This data is listed in Table 6 and illustrated in Fig. 7.

Table 6. Comparison of JSC and PE Mass Contained in Mortar Samples and PE Released into Water

Samples	JSC Mass in Each Sample (g)	PE Mass in Each Sample (mg)	Released PE Mass (mg)	Standard Deviation (mg)	Amount Released (%)
MJSC2	5.00	6.37	0.28	0.117	4.39
MJSC4	10.00	12.75	0.40	0.038	3.14
MJSC6	15.00	19.12	0.34	0.130	1.78
MJSC8	20.00	25.49	0.63	0.268	2.47
MJSC10	25.00	31.87	0.73	0.435	2.29

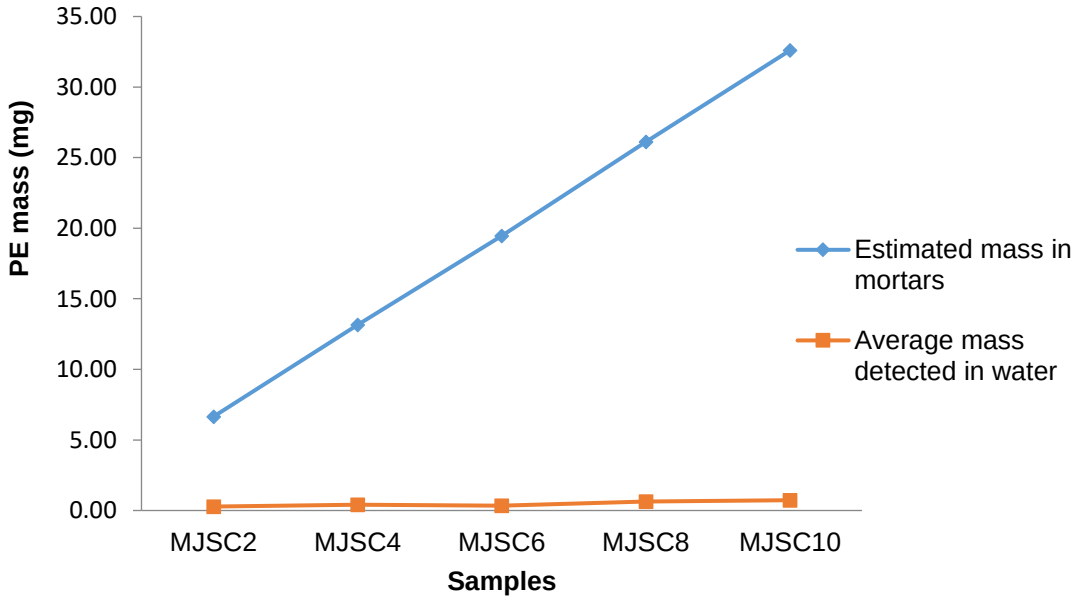

Fig. 7. Content of PE released by mortars containing JSC

Figure 7 indicates that the amount of PE released by the hardened mortars remained constant even when the PE content increased as a function of the increase in JSC content. The mass of PE released by the mixture did not exceed 5 mg, even in the case of the mortar sample with the highest JSC content, which had a PE content of more than 30 mg. The probable reason for this event is that the toxin was stabilized inside the specimen, this portion of phorbol due to the release of toxins from the surface of the sample. Therefore, although by the compressive strength achieved there may be several applications for concrete, the JSC increase in concrete will be preferable in buildings where the mortar does not contain JSC in its mix in order to cubrir the surface of the concrete, as a structural block of masonry among other applications with mortar.

CONCLUSIONS

1. The axial compression test revealed that the addition of *Jatropha* seed cake (JSC) had a stronger impact on the strength of the mortar than of that on the concrete. The axial compressive strength of concrete composition containing 2% of JSC in place of sand decreased 22, 10, and 10% at 7, 28, and 90 days, respectively and the composition containing 4% of JSC in place of sand decreased 27, 34, and 21%, respectively. Concrete CJSC2 showed that the decrease of resistance, relative to the reference concrete, decreased with time. The concrete with the largest amount of JCS (CJSC4) only showed this drop for the 90 days. This must have occurred due to the fact that the mortar has no gravel. The presence of gravel increases the strength of the concrete and is even increased by JSC, which reduces the strength of the same. Thus, the concrete was less sensitive to the addition of 2 and 4% of JSC than the mortar.
2. The HPCL results confirmed the excellent performance of the cementitious matrix in rendering the toxin inside it inert, as very little phorbol ester (PE) was released into the water compared with the amount of PE inserted into the mortars in the casting process. The small amounts of PE found in the water can be explained by the release of PE molecules located on the surface of the test specimens. Therefore, the increase of JSC should be in the concrete and its surface should be covered with mortar without JSC, although the MJSC2 mortar achieved a suitable compression strength for use in non-structural walls.
3. In this context, the inclusion of toxic organic compounds in the cementitious matrix is an important way to protect against, and immobilize these compounds . Knowledge of ways to render toxic material inert including it in the formulation of concrete is essential because it should enable concretes, in the future, to be correlated with solutions for protection against toxins contained in well known hazardous products, such as aerosols and pesticides.

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