New recycling process for the foundry sands: innovation aimed to get materials with high added value

Action C Methods and process to transform the scraps in new products

Task C4 Chemical and bio tests

Sub-actions C4.1 Screening of the leaching solutions, C4.2 Correlation between different concentrations and biological effect

Final report

Table of contents

Introduction

To assess the environmental impact of materials, a useful tool is provided by ecotoxicological test based on different target organisms, selected on the ecological representativeness. The recommended tests battery for liquid samples or leachate of solid samples is composed of three organisms: bacteria *Allivibrio fischeri*, microalgae *Pseudokirchneriella subcapitata* and crustacean *Daphnia magna*. The purpose of this action was to assess the effect of leaching solutions (produced according to EN 12457-2) on the selected organisms.

Material and methods

Foundry plants

The 39 foundry plants involved in this study were interviewed using an *ad hoc* questionnaire designed to gather information on the casting processes, including details about metal cast, original sands, and binding systems. Samples of sands were collected between May and August 2021.

Following an initial screening based on the physical-chemical characterization obtained in Task C3, 25 samples were selected for the ecotoxicological assessment.

Leaching tests

Leaching tests were performed on samples according to the European regulation for the characterization of waste (EN 12457-2 2002). Tests were performed by mixing the homogenized samples with demineralized water (pH 7) at a liquid to solid ratio of 10 L/kg to obtain eluates of nominal concentration of 100 g/L (the concentration is equivalent to the extracted compounds of originally 100 g sample per litre). To study eluates at different concentrations of sample, the leaching tests were also performed at higher liquid to solid ratio (20, 40, 60, 120 L/kg) obtaining eluates of nominal concentration of 50, 25, 12.5, 6.25 g/L. The mixtures were placed on a tightly closed rotary shaker and agitated for 24 h, rotating at 10 ± 2 rpm (Figure 1A). The solutions were filtered through 0.45 μm filters (Figure 1B).

Figure 1 - Leaching test. Rotating (A) and filtering (B) apparatus.

The pH and the electric conductivity (EC) were measured with a multiparameter instrument equipped with pH and conductivity probes (EDGE, Hanna Instruments).

Toxicity tests

Microtox toxicity assay

The toxicity towards the bioluminescent bacteria *Allivibrio fischeri* was measured using the Microtox toxicity assay, according to the ISO 11348-3 (EN ISO 11348-3 2018) (Figure 2).

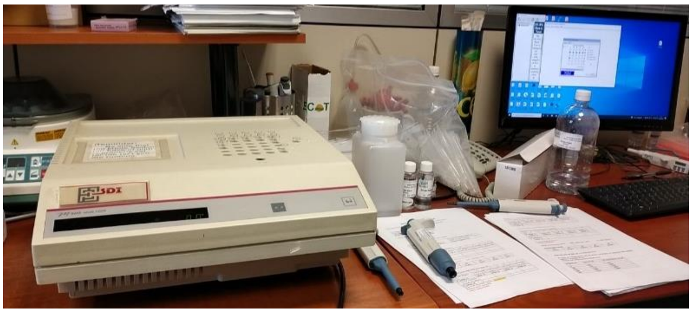

Figure 2 - Microtox apparatus.

In a first step, the 81.9% Screening Test was carried out on all undiluted leachates and their toxicity was evaluated. The second step of the analysis was a test with dilutions (81.9% Basic Test). For each toxic sample, five serial dilutions (50, 25, 12.5, 6.25 g/L) were tested. The negative control was the Microtox diluent (NaCl 2%). The luminescence decrease was evaluated after 5, 15 and 30 min of exposure by using a Microbics Model 500 Toxicity Analyzer and Calculations were performed using the program MicrotoxOmni according to the manufacturer's instructions (Microbics Corporation). The results were expressed as luminescence inhibition percentage with respect to the control and when it was possible as EC50.

Daphnia magna acute immobilization test

The assay was conducted by using Daphtoxkits F (Ecotox LDS), according to the standard procedure (UNI EN ISO 6341 2013). 20 neonates of *D. magna* (<24-h-old) were used for each tested condition (Figure 1A-B). As a first step, a screening test was performed. The leachates were tested undiluted (nominal concentration 100 g/L) without any modification (pH or salts) (Figure 3C). Dilutions (50, 25, 12.5, 6.25 g/L) were subsequent tested to assess the lowest toxic concentration (Figure 3D).

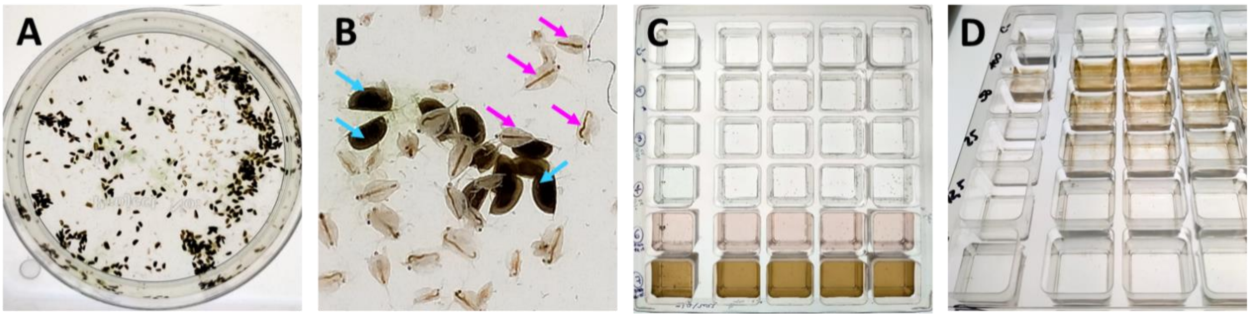

Figure 3 - *Daphnia magna* immobilization test. Neonates of *D. magna* (pink arrows) hatched from dormant eggs (ephippia, cyan arrows) (A and B). Screening test (C) and dilutions test (D).

Effects on crustacean movements or death were observed after 24 and 48 h of contact. Standard freshwater was used as a negative control in every test. Experiments were performed in duplicate. The percentage of immobilized animals was determined, and the half maximal effective concentration (EC50) values were calculated by probit regression with the confidence interval (CI) set at 95%.

Pseudokirchneriella subcapitata growth inhibition test

The *Pseudokirchneriella subcapitata* growth inhibition assay was conducted following the ISO 8692 (UNI EN ISO 8692 2012) by using Algaltoxkit F (Ecotox LDS) (Figure 4A). A mini-scale test method was applied (Figure 4B).

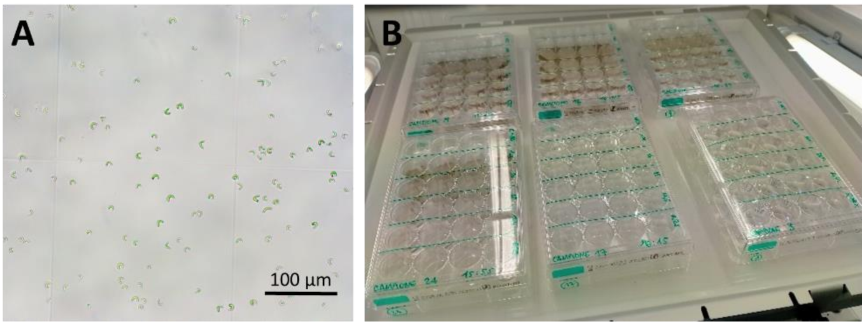

Figure 4 - *Pseudokirchneriella subcapitata* cell suspension (A) and the mini-scale inhibition test setting (B).

The initial algal density was 10^4 cells/mL in 2 mL of each sample at the concentration of 100 g/L, adjusted to culturing condition with concentrated nutrient solutions. The samples were incubated for 72 h with orbital agitation (100 rpm), at 23 ± 1 °C and at 10000 lux. Dilutions of eluates (50, 25, 12.5, 6.25 g/L) were tested to assess the lowest toxic concentration Algal growth medium was used as a negative control. The test was performed in quadruplicate. The algal growth rates were calculated based on the daily reading of optical density at 690 nm, if possible, alternatively on the daily manual counting by FastRead 102 counting chamber (Biosigma, Italy). The cellular density was determined through a 6 points standard curve (from 1×10^4 to 1×10^7 cells/mL). The assay is considered valid if the average growth rate in the control is at least 1.4/day and the variation coefficient of the growth rate in the control replicates do not exceed 5%. Toxicity was expressed as the percentage of growth inhibition (I%) in comparison with the control, and when it was possible as EC50 calculated by probit regression with the confidence interval (CI) set at 95%. The statistical analysis was conducted by using the Student's *t* test.

Toxicity classification systems

To obtain a comprehensive evaluation of the tested samples, an integrated toxicity classification approach was implemented. Three different systems were applied and compared.

The "Toxicity Classification System", proposed by Guido Persoone and colleagues (Persoone et al. 2003) was developed for the toxicity classification of waters or wastewaters, industrial effluents, soil and waste dump leachates. The scoring system ranks the samples in 5 classes of increasing hazard/toxicity, with calculation of a weight factor for the concerned hazard/toxicity class (Table 1).

Table 1 - Toxicity Classification System (Persoone et al. 2003)

Class	Toxicity	Symbol
I	No acute toxicity	☺
II	Slight acute toxicity	☹
III	Acute toxicity	☠
IV	High acute toxicity	☠☠
V	Very high acute toxicity	☠☠☠

The “Toxicity test Battery integrated Index”, described by Sonia Manzo and colleagues (Manzo et al. 2014), was proposed for the screening of the ecotoxicological risk of sediment elutriates, pore waters, and sediment suspensions in different marine ports. The scoring system ranks the samples in 5 classes of increasing ecotoxicological risk level (Table 2).

Table 2 - Toxicity test Battery integrated Index (Manzo et al. 2014)

TBI (%)	C	Ecotoxicological risk level	
≤5		Absent	☺
5<>20	≤0	Low	☹
5<>20	>0	Medium	☠
20<>50		High	☠☠
>50		Very high	☠☠☠

The “Ecoscore System”, defined by Christine Lors and colleagues (Lors, Ponge, and Damidot 2018), was applied to assess the environmental hazard of PAH-polluted soils. The system classifies the samples in four levels of toxicity (Table 3).

Table 3 - EcoScore system (Lors, Ponge, and Damidot 2018)

EcoScore	Intensity of toxicity
0	No
0<ES≤33	Weak
33<ES≤67	Moderate
67<ES≤100	Strong

Results

Characteristics of the foundry sands

The data collected from the questionnaires showed that 76% of the foundries produce castings in cast iron, 16% in steel and 8% in copper alloys. The main sand used is silica sand, coming from France, Italy, and Portugal. Eight foundries use the "green" moulding process, nine the "resin" moulding process, while five apply both types. In the case of “green” moulding, the sand activation processes to obtain the moulds involve the use of bentonite and mineral black, while in the case of “resin” moulding, phenolic, furan or isocyanate agglomerates or additives are used. The cores, the production of which requires only "resin" moulding, are obtained by different processes depending on the aggregates or additives and the catalysts added (Table 4).

Table 4 - Characteristics of the sand's samples selected.

Sample #	Metals cast	Sands (origin)	Binding system			
			Green	**Resin**	**Core**	**Shell**
1	Iron	Silica (Portugal, France)		X	Sodium silicate - ester cured	Sodium silicate - ester cured
3	Iron	Silica (Portugal)		X	Furan no-bake	Furan no-bake
4	Iron	Silica (Portugal)		X	Furan no-bake	Furan no-bake; Ashland; Shell Molding
6	Steel	Silica, zirconium, chromite, cerabeads		X	Sodium silicate - ester cured	Phenolic/urethane no-bake; Ashland; Shell Molding
7	Steel	Silicea, chromite	X		Bentonite	Phenolic/urethane no-bake
9	Iron	Carbomix, american bentonite, silica (France)	X		Bentonite	n.u.
10	Iron	Silica	X		Bentonite	Ashland
11	Steel	Silica, chromite		X	Sodium silicate - ester cured	Sodium silicate - ester cured; Phenolic/urethane no-bake
12	Copper	Silica (Portugal, France), chromite	X	X	Bentonite; Sodium silicate - ester cured	Phenolic no-bake; Phenolic/urethane no-bake
14	Iron	Silica (France, Italy)	X	X	Furan no-bake; Phenolic no-bake	Furan no-bake, Phenolic no-bake; Ashland; Shell Molding
15	Iron	Silica (France)		X	Furan no-bake	Furan no-bake
16	Iron	Silica (Italy)	X		Bentonite	n.u.
17	Iron	Silica (Portugal)		X	Furan no-bake	Furan no-bake
18	Copper	Silica (Francia)	X	X	Sodium silicate - ester cured	Furan no-bake; Ashland; Shell Molding
24	Iron	Silica	X		Bentonite	n.u.
26	Iron	Silica	X		Bentonite	n.u.
27	Iron	Silica (Italy, France), chromite	X	X	Bentonite; Furan no-bake	Furan no-bake
28	Iron	n.d.	n.d.	n.d.	n.d.	n.d.
29	Iron	Silica (France), chromite	X	X	Bentonite	Furan no-bake; Phenolic/urethane no-bake
30	Iron	Silica (France)	X	X	Bentonite; Furan no-bake	n.u.
31	Steel	n.d.	n.d.	n.d.	n.d.	n.d.
32	Iron	Silica (Italy)	X		n.u.	Bentonite; Ashland; Shell Molding
34	Iron	Silica		X	Furan no-bake	Furan no-bake
35	Iron	Silica (Italy)		X	Shell Molding	Shell Molding
39	Iron	Silica (Portugal)	X		Bentonite	Furan no-bake; Phenolic no-bake

n.u.: not used; n.d.: not declared

The dry weight and moisture values of each sample were determined (Table 5). These values ranged from 85.38% to 99.89% and from 0.11% to 14.62% respectively.

Table 5 - Dry weight and moisture content of the foundry sands samples.

Sample #	Dry weight (%)	Moisture (%)
1	98.7	1.3
3	97.2	2.8
4	95.2	4.8
6	99.3	0.7
7	99.4	0.6
9	98.8	1.2
10	97.6	2.4
11	99.5	0.5
12	99.9	0.1
14	96.5	3.5
15	85.4	14.6
16	99.3	0.7
17	99.8	0.2
18	97.9	2.1
24	98.1	1.9
26	96.5	3.5
27	99.5	0.5
28	98.8	1.2
29	97.0	3.0
30	98.5	1.5
31	99.8	0.2
32	96.1	3.9
34	99.1	0.9
35	99.8	0.2
39	87.9	12.1

Characteristics of the leachates of sands

Table 6 shows the analysis carried out on eluates produced by leaching tests (UNI EN 12457-2) to allow the recovery of the sands, according to the Italian legislation for the recovery of non-hazardous waste (Ministerial Decree n. 186 2006), supplied by nine foundries participating in the study (#2, 4, 8, 9, 11, 16, 18, 28, 34, 39). The release of pollutants was generally below the limit values, with many parameters even below the quantification limit of the analyses. However, sample 4 was characterized by several parameters out of the limit values: fluorides, copper, nichel and COD values were strongly higher than the limits, as well as the pH was lower of the permitted range. Slight exceedances of the limits were also observed in sample 16 for the fluorides and COD values (2.7 and 50 mg/L, respectively). COD limit was surpassed also by samples 18 and 39 (48 and 37.7 mg/L).

Table 6 - Chemical characterisation of eluates of sands intended for recovery.

Parameters	**MU**	**Limit value MD 186/2006**	**Sample #**									
			2	**4**	**8**	**9**	**11**	**16**	**18**	**28**	**34**	**39**
Chlorides	mg/L	100	3	12.2	2.48	7,1	3.38	20	10.2	22	75.3	4.4
Nitrates	mg/L	50	3	<q.l.	<q.l.	2	2.73	2	4.1	20	29.8	0.22
Fluorides	mg/L	1.5	1.3	14.7	<q.l.	1.4	<q.l.	2.7	1.38	1.3	0.25	3.8
Sulphates	mg/L	250	3	184	<q.l.	34.5	25.9	221	29.5	84	68.2	81
Cyanides	µg/L	50	<q.l.	<q.l.	<q.l.	<q.l.	<q.l.	<q.l.	<q.l.	<q.l.	<q.l.	<q.l.
Barium	mg/L	1	0.017	0.06	0.002	0.09	<q.l.	0.07	0.028	0.04	0.045	0.008
Copper	mg/L	0.05	0.003	0.077	0.023	0.002	<q.l.	0.005	0.038	q.l.	<q.l.	0.003
Zinc	mg/L	3	0.039	2.55	0.084	0.01	<q.l.	0.016	0.999	0.02	0.084	<q.l.
Beryllium	µg/L	10	<q.l.	0.5	<q.l.	<q.l.	<q.l.	<q.l.	2	<q.l.	<q.l.	<q.l.
Cobalt	µg/L	250	<q.l.	44	<q.l.	<q.l.	<q.l.	<q.l.	19	<q.l.	<q.l.	<q.l.
Nichel	µg/L	10	<q.l.	219	<q.l.	<q.l.	<q.l.	2	5	<q.l.	<q.l.	<q.l.
Vanadium	µg/L	250	19.9	1.16	<q.l.	20.1	<q.l.	13	64	<q.l.	<q.l.	<q.l.
Arsenic	µg/L	50	1.2	<q.l.	1.07	11.2	<q.l.	7	4	<q.l.	<q.l.	0.7
Cadmium	µg/L	5	<q.l.	3.47	<q.l.	<q.l.	<q.l.	<q.l.	0.5	<q.l.	<q.l.	<q.l.
Total Chromium	µg/L	50	6.28	1.8	<q.l.	2.3	<q.l.	<q.l.	13	<q.l.	<q.l.	<q.l.
Lead	µg/L	50	4.96	2.36	<q.l.	<q.l.	<q.l.	4	33	<q.l.	<q.l.	<q.l.
Selenium	µg/L	10	<q.l.	q.l.	<q.l.	1.5	<q.l.	4	5	<q.l.	<q.l.	<q.l.
Mercury	µg/L	1	<q.l.	q.l.	<q.l.	<q.l.	<q.l.	<q.l.	0.2	<q.l.	<q.l.	<q.l.
Asbestos	mg/L	30	<q.l.	q.l.	<q.l.	<q.l.	n.d.	n.d.	<q.l.	n.d.	<q.l.	<q.l.
COD	mg/L	30	23	792	<q.l.	28	<q.l.	50	48	<q.l.	23.7	37.7
pH	-	5.5<>12	8.5	4.66	6.21	9.36	8.29	9.2	6.09	6.5	6.07	8.63
Conductivity	µS/cm	-	101	n.d.	n.d.	421	n.d.	669	n.d.	n.d.	n.d.	349

MU: measure unit; COD: chemical oxygen demand; q.l.: quantification limit; n.d.: not determined; grey background: value above the limit

In Table 7 are summarized the analysis carried out on eluates produced by leaching tests (UNI EN 12457-2) to allow the landfill disposal of the sands, according to the Italian legislation for the landfill disposal (Ministerial Decree 2010), supplied by seven foundries participating in the study (#4, 6, 9, 10, 16, 26, 34, 39). Even for this analytical setting, the release of pollutants was generally below the limit values, with many parameters even below the quantification limit of the analyses. However, sample 4 was characterized by two parameters out of the limit values: fluorides and COD values were higher than the limits (15.4 and 228 mg/L, respectively). The last value higher than the limits, was the COD of sample 34.

Table 7 - Chemical characterisation of eluates of sands intended for landfill disposal.

Parameters	MU	Limit value MD 27/09/2010	Sample #							
			4	6	9	10	16	26	34	39
Chlorides	mg/L	2500	12.8	9.2	13.3	12.6	20	3.91	<q.l.	5,3
Fluorides	mg/L	15	15.4	4.1	3.1	2.9	2.7	0.64	<q.l.	2.57
Sulphates	mg/L	5000	193	8.4	41.8	27.5	221	71	198	53
Antimuonium	mg/L	0.07	<q.l.	0.001	0.001	0.001	0.002	0.001	<q.l.	<q.l.
Barium	mg/L	10	0.065	0.046	0.786	0.188	<q.l.	0.045	<q.l.	0.01
Copper	mg/L	5	0.077	0.026	0.009	0.04	<q.l.	0.005	<q.l.	<q.l.
Zinc	mg/L	5	2.58	0.027	0.071	0.243	<q.l.	0.017	0.9	<q.l.
Molybdenum	mg/L	1	<q.l.	0.027	0.028	0.004	0.022	0.54	<q.l.	0.006
Nichel	µg/L	1000	220	4.8	5.4	4.38	<q.l.	31.0	300	1.66
Arsenic	µg/L	200	<q.l.	3.75	11	3.74	7	10.2	<q.l.	<q.l.
Cadmium	µg/L	100	3.32	<q.l.	<q.l.	<q.l.	<q.l.	0.250	<q.l.	<q.l.
Total Chromium	µg/L	1000	2.08	36	2.4	<q.l.	<q.l.	5.42	<q.l.	<q.l.
Lead	µg/L	1000	2.96	2.5	11	13.3	<q.l.	2.11	<q.l.	<q.l.
Selenium	µg/L	50	<q.l.	<q.l.	1.4	2.35	<q.l.	1.66	<q.l.	<q.l.
Mercury	µg/L	20	<q.l.	<q.l.	<q.l.	<q.l.	<q.l.	0.101	<q.l.	<q.l.
DOC	mg/L	100	228	n.d.	26	32	16.8	10.0	143	34
pH	-	-	4.66	10.2	9.68	10.2	9.2	9.9	3.9	8.67
Conductivity	µS/cm	-	n.d.	810	410	357	669	462	n.d.	329
TDS	mg/L	10000	n.d.	750	1090	1320	440	356	230	145

MU: measure unit; DOC: dissolved organic carbon; TDS: total dissolved solids; q.l.: quantification limit; n.d.: not determined; grey background: value above the limit

Characteristics of the leachates of studied samples

Leachates were obtained from 25 samples of foundry sands. The nominal concentration of the undiluted leachates was 100 g/L. Fourteen of them (56% of total) were coloured, from slight tinting (samples #6, 11, 14, 26, 28) to a darker brown colouring (samples #7, 9, 10, 16, 18, 24, 29, 30, 31) (Figure 5).

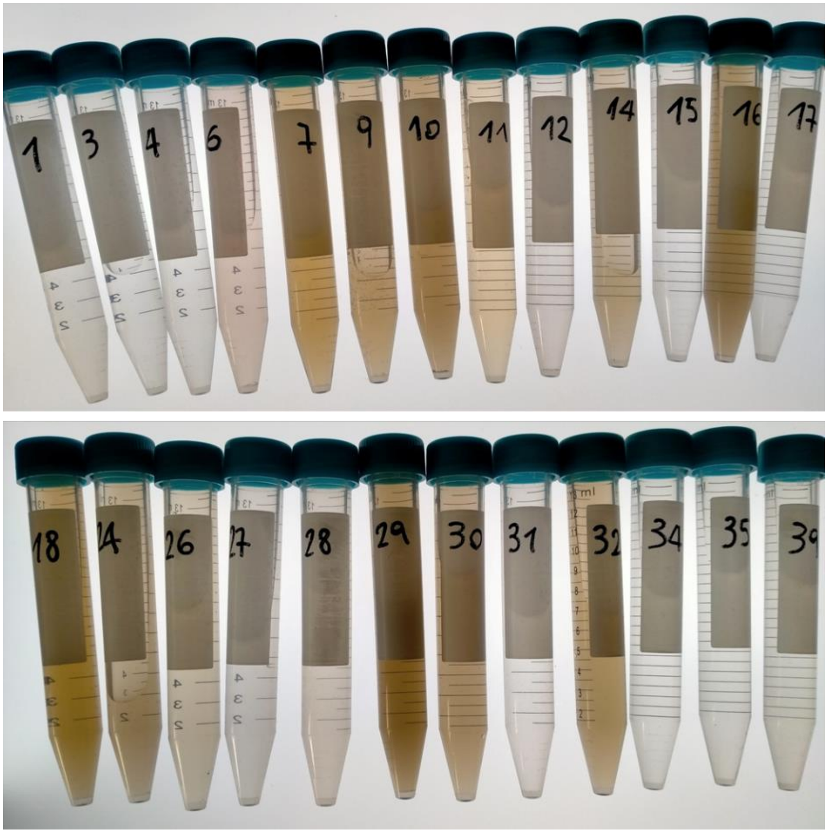

Figure 5 - Leachates obtained from the foundry sands according to EN 12457-2.

The pH and the electric conductivity (EC) values of the leachates determined in the study are summarized in Table 8.

Table 8 - pH and electric conductivity (EC) values of eluates.

Sample #	pH	EC (µS/cm)
1	8	510
3	6.5	623
4	6	433
6	8	1756
7	7	332
9	7.5	396
10	7	392
11	8	1290
12	7	132.6
14	7	526
15	5	717
16	7	291
17	7	522
18	8	348
24	9.5	417
26	7	886
27	7	291.1
28	7	388
29	9	507
30	8	341
31	7	92.4
32	7	415
34	5	483
35	7	12.5
39	7	190.5

The pH ranged from 5 (#34) to 9.5 (#24), with a median value of 7. The EC values were highly variable, ranging from 12.5 (#35) to 1756 (#6) µS/cm, and a median value of 415 µS/cm (Figure 6).

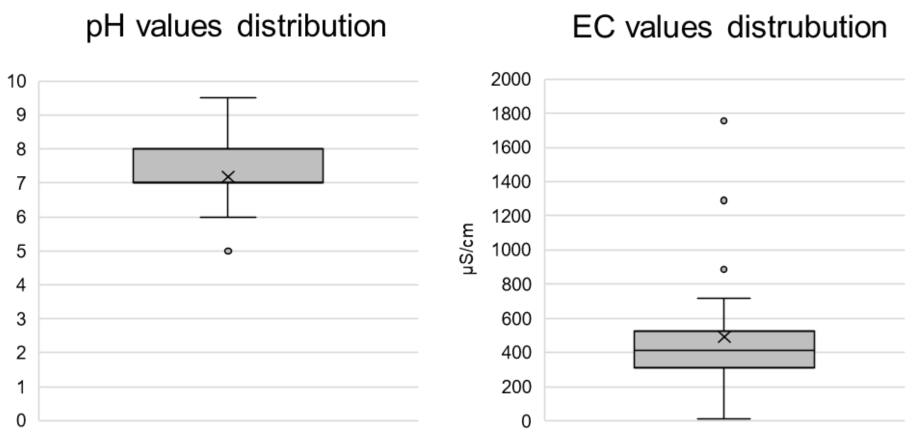

Figure 6 - pH and EC values distributions.

Assessment of biological effects of leachates

The solutions obtained from leaching tests of 25 foundry sands were assayed by using ecotoxicity tests on bacteria (*Allivibrio fischeri*), animals (*Daphnia magna*) and algae (*Pseudokirchneriella subcapitata*).
The solutions were tested undiluted (nominal concentration 100 g/L), and, in the case of toxicity, five dilutions were analysed to calculate the EC50 (Half maximal effective concentration, the concentration of a compound where 50% of the population exhibit a response).

The Microtox screening test revealed that the majority (18/25, 72%) of undiluted samples was not toxic. Six samples acted as biostimulator (#3, 17, 28, 31, 34, 35), 4 samples generated negligible inhibition of bioluminescensce (less than 10%) (#12, 15, 27, 39), while 8 samples caused slight effect (about 10%) (#9, 10, 14, 16, 24, 29, 30, 32). On the other hand, 6 samples (#1, 4, 6, 11, 18, 26), corresponding to the 24% of tested samples, showed a strong inhibition of bioluminescensce (above 30%). Samples 6 and 11 caused the grater effect (100% of inhibition), while the others samples (#1, 7, 18, 26) generated an inhibition ranging form 60 to 30% (Figure 7).

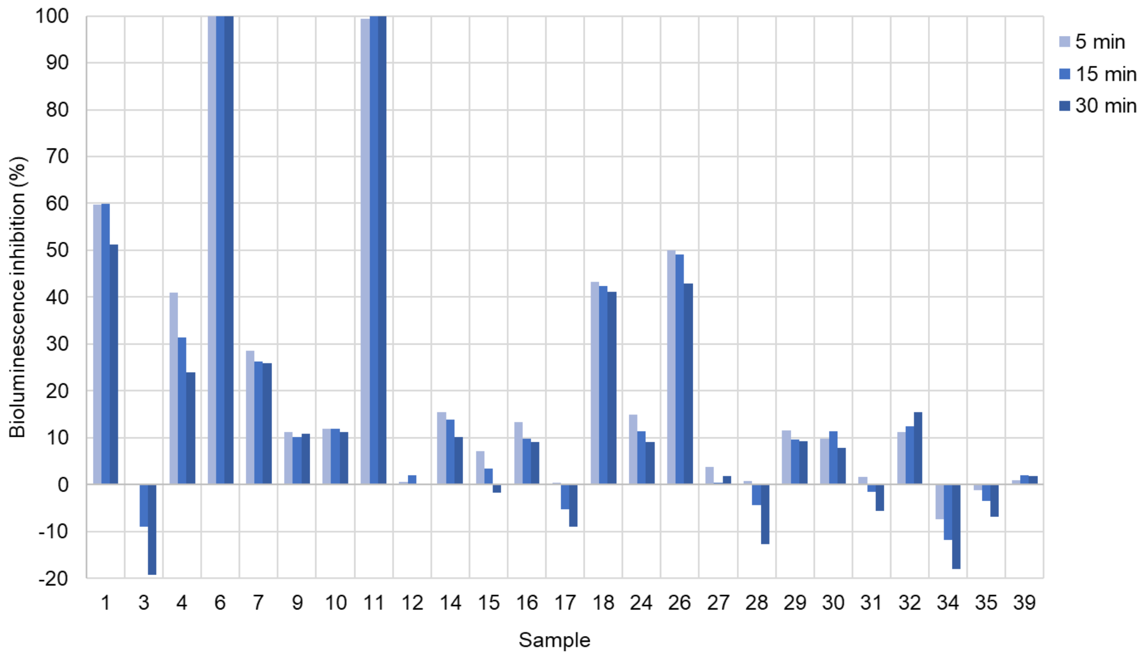

Figure 7 - *Allivibrio fischeri* bioluminescence screening test on undiluted leachates (nominal concentration 100 g/L).

These toxic samples were subjected to further analysis to enabled the determination of EC50 values after 30 minutes (Table 9). Samples 6 and 11 comfirmed the highest toxicity, with the lowest EC50 values (<5 and 6.6 g/L, respectively).

Table 9 - EC50 values determined by the *Allivibrio fischeri* bioluminescence inhibition test.

Sample #	EC_{50}30min (g/L)
1	31.6 (19.8-50.2)
4	50.0 (24.5-102.3)
6	<5
11	6.6 (6.3-6.8)
18	95.4 (64.5-123.1)
26	97.7 (73.1-103.7)

>: EC50 greater than the highest concentration tested

Comparably, the *Daphnia magna* immobilization test on undiluted samples demonstrated that the majority (16/25, 64%) was clearly not toxic and only only a third of the samples increased the immobilization above 10% (9 samples, 36%, #4, 6, 7, 10, 11, 12, 14, 15, 31) (Figure 8).

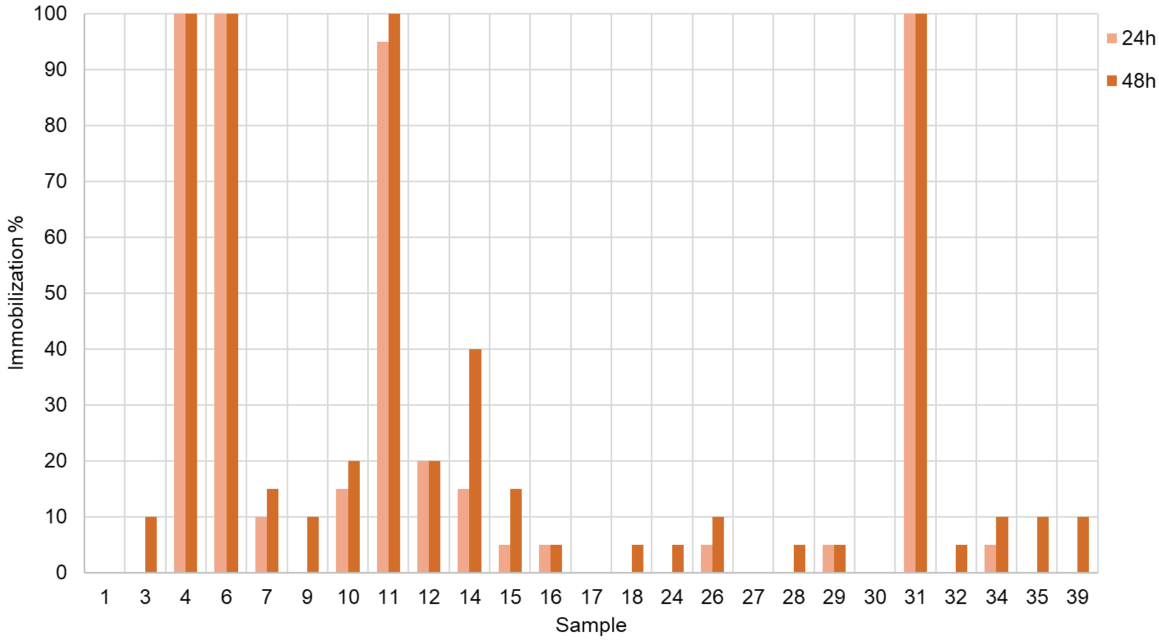

Figure 8 - *Daphnia magna* immobilization screening test on undiluted leachates (nominal concentration 100 g/L).

The toxic samples were subjected to further analysis to determined the EC50 values after 48 hours (Table 10). For 3 samples (#12, 15, 31) the EC50 values were greater than the highest concentration tested (100 g/L). Samples 7, 10 and 14 were the more toxic, with the lowest EC50 values (17.7, 13.3 and 5.6 g/L, respectively).

Table 10 - EC50 values determined by the *Daphnia magna* immobilization test.

Sample #	EC_{50}48ore (g/L)
4	57.0 (26.8-369.9)
6	29.6 (19.2-43.5)
7	17.7 (13.9-22.3)
10	13.3 (13.0-86.6)
11	44.7 (32.1-60.2)
12	>
14	5.6 (0.3-10.9)
15	>
31	>

>: EC50 greater than the highest concentration tested

The *Pseudokirchneriella subcapitata* growth inhibition test appears as the most sensitive assay of the battery, as clearly emphasized by Figure 9. Indeed, differently from the above-described tests, the majority (20/25, 80%) of undiluted samples strongly affected the algal growth.

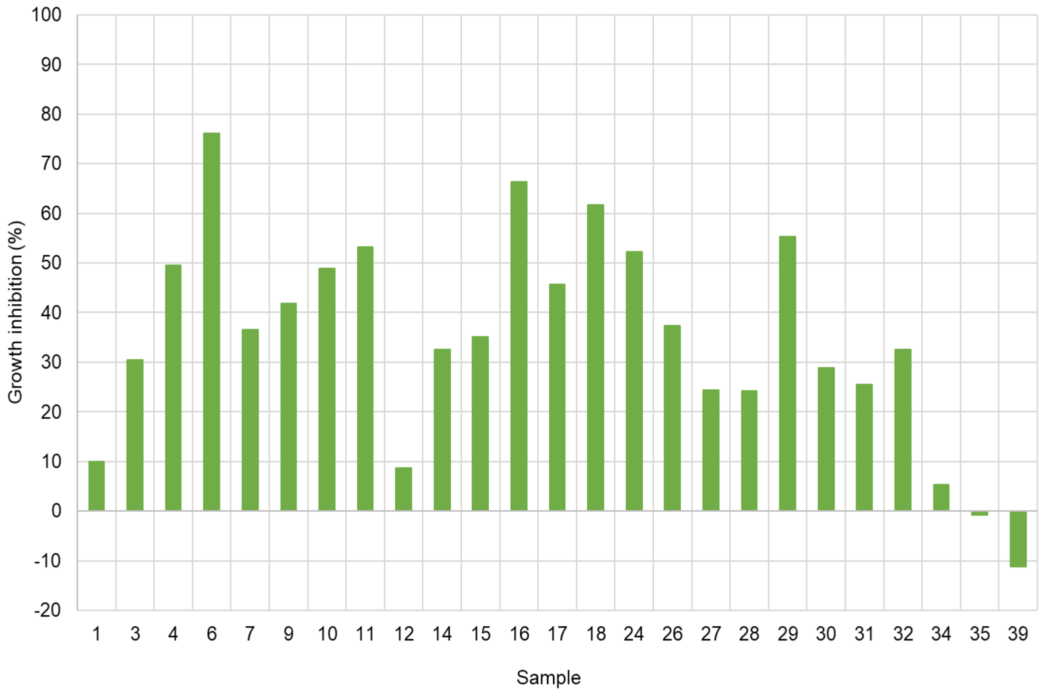

Figure 9 - *Pseudokirchneriella subcapitata* growth inhibition screening test on undiluted leachates (nominal concentration 100 g/L).

The toxic samples were subjected to further analysis to enabled the determination of EC50 values after 72 hours (Table 11). For 12 samples (63% of the toxic samples, 48% of the total, #3, 4, 9, 16, 17, 24, 26, 27, 28, 30, 31, 32) the EC50 values were greater than the highest concentration tested (100 g/L). Five samples (26%, 20 % of the total, #6, 11, 15, 18, 29) showed an EC50 ranging from 89.1 to 31.6 g/L. Two samples (#10 and 14) presented the highest toxicity, with the lowest EC50 values (8.4 and 2.5 g/L, respectively).

Table 11 - EC50 values determined by the *Pseudokirchneriella subcapitata* growth inhibition test.

Sample #	EC_{50}72ore (g/L)
3	>
4	>
6	31.6 (28.8-34.3)
9	>
10	8.4 (7.5-10.5)
11	33.1 (27.4-41.6)
14	2.5 (0.0-4.9)
15	74.8 (52.4-105.3)
16	>
17	>
18	69.0 (49.8-116.4)
24	>
26	>
27	>
28	>
29	89.1 (76.9-106.7)
30	>
31	>
32	>

>: EC50 greater than the highest concentration tested

To enable a comprehensive interpretation of the resulted toxicity, the data were integrated through different toxicity scores. The toxicity score is a useful instrument for providing responses in regulatory and management frameworks because it allows classification, synthesis, and easy visualization of toxicity data. Different research groups recently proposed classifications of toxicity by comparing biological responses. In this study three system were compared: the Toxicity Classification System (TCS - Persoone et al. 2003), the Toxicity test Battery integrated Index (TBI - Manzo et al. 2014) and the EcoScore system (EC - Lors et al. 2018). These systems are based on different parameters for the analysis of different matrices (waters or wastewaters, industrial effluents, soil, waste dump leachates, sediment elutriates).

Undiluted samples were ranked according to all the three applied systems, as summarized in Table 12.

Table 12 - Ranking of the undiluted samples (nominal concentration 100 g/L) according to the Toxicity Classification System (TCS - Persoone et al. 2003), the Toxicity test Battery integrated Index (TBI - Manzo et al. 2014) and the EcoScore system (EC - Lors et al. 2018).

Toxicity Classification System					Toxicity test Battery integrated Index					EcoScore System		
Sample	**Hazard Class**	**Class weight score %**	**Hazard**		**Sample**	**TBI%**	**C**	**Ecotoxicological risk level**		**Sample**	**EcoScore**	**Intensity of toxicity**
12	I	0.0	No acute	☺	**34**	-1.2		Absent	☺	**34**	0	No tox
34	I	0.0	No acute	☺	**28**	-0.8		Absent	☺	**35**	0	No tox
35	I	0.0	No acute	☺	**39**	-0.8		Absent	☺	**39**	0	No tox
39	I	0.0	No acute	☺	**35**	-0.2		Absent	☺	**12**	22	Weak
3	II	33.3	Slight acute	☹	**3**	1.9		Absent	☺	**3**	22	Weak
9	II	33.3	Slight acute	☹	**12**	3.2		Absent	☺	**17**	22	Weak
15	II	33.3	Slight acute	☹	**27**	3.4		Absent	☺	**24**	22	Weak
17	II	33.3	Slight acute	☹	**30**	4.8		Absent	☺	**27**	22	Weak
27	II	33.3	Slight acute	☹	**15**	4.9		Absent	☺	**28**	22	Weak
28	II	33.3	Slight acute	☹	**17**	5.8	3.4	Medium	☠	**29**	22	Weak
30	II	33.3	Slight acute	☹	**31**	6.1	3.4	Medium	☠	**30**	22	Weak
31	II	33.3	Slight acute	☹	**32**	6.1	3.4	Medium	☠	**1**	33	Weak
32	II	33.3	Slight acute	☹	**9**	6.9	3.4	Medium	☠	**9**	33	Weak
10	II	66.7	Slight acute	☹	**1**	7.9	3.4	Medium	☠	**15**	33	Weak
14	II	66.7	Slight acute	☹	**24**	8.2	3.4	Medium	☠	**16**	33	Weak
26	II	50.0	Slight acute	☹	**29**	8.7	3.4	Medium	☠	**32**	33	Weak
1	III	33.3	Acute	☠	**26**	9.5	3.4	Medium	☠	**26**	39	Moderate
16	III	33.3	Acute	☠	**14**	9.6	3.4	Medium	☠	**10**	44	Moderate
24	III	33.3	Acute	☠	**16**	10.2	3.4	Medium	☠	**7**	56	Moderate
29	III	33.3	Acute	☠	**10**	12.5	3.4	Medium	☠	**14**	56	Moderate
7	III	55.6	Acute	☠	**18**	12.8	3.4	Medium	☠	**18**	56	Moderate
18	III	66.7	Acute	☠	**7**	21.0	3.4	Medium	☠	**31**	56	Moderate
4	IV	55.6	High acute	☠☠	**4**	22.7	3.4	Medium	☠	**4**	78	Strong
6	IV	88.9	High acute	☠☠	**11**	34.1		High	☠☠	**11**	89	Strong
11	IV	88.9	High acute	☠☠	**6**	37.3		High	☠☠	**6**	100	Strong

Three samples (#34, 35, 39) were recognized not toxic by all the applied classification systems. On the other side of the toxicity rank, samples 6 and 11 were classified highly toxic according the three systems. Sample 4 was filed in the last class by two systems (TCS and ES) and just above the threshold of "high toxicity" by the TBI system. Most of the samples were differently classified in intermediate positions (identify with "slight" or "acute", "medium" and "weak" or "moderate").

Regarding the classification of the undiluted samples through the TCS (Figure 10A), 4 samples (16%) were non-hazardous, 12 samples (48%) were slightly hazardous, 6 samples (24%) were hazardous, and 3 samples

(12%) were highly hazardous. None of the samples was allocated in the last class of the system ("Very high acute hazard").

As a result of the TBI classification (Figure 10B), 9 samples (36%) were non-toxic, 14 samples (56%) were slightly hazardous, 6 samples (24%) were hazardous, and 3 samples (12%) were highly hazardous. None of the samples was allocated in the last class of the system ("Very high acute hazard").

The EC system (Figure 10C) classified 3 samples (12%) as non-toxic, 13 samples (52%) as weakly toxic, 6 samples (24%) with a moderate toxicity, and 3 samples (12%) as strongly toxic.

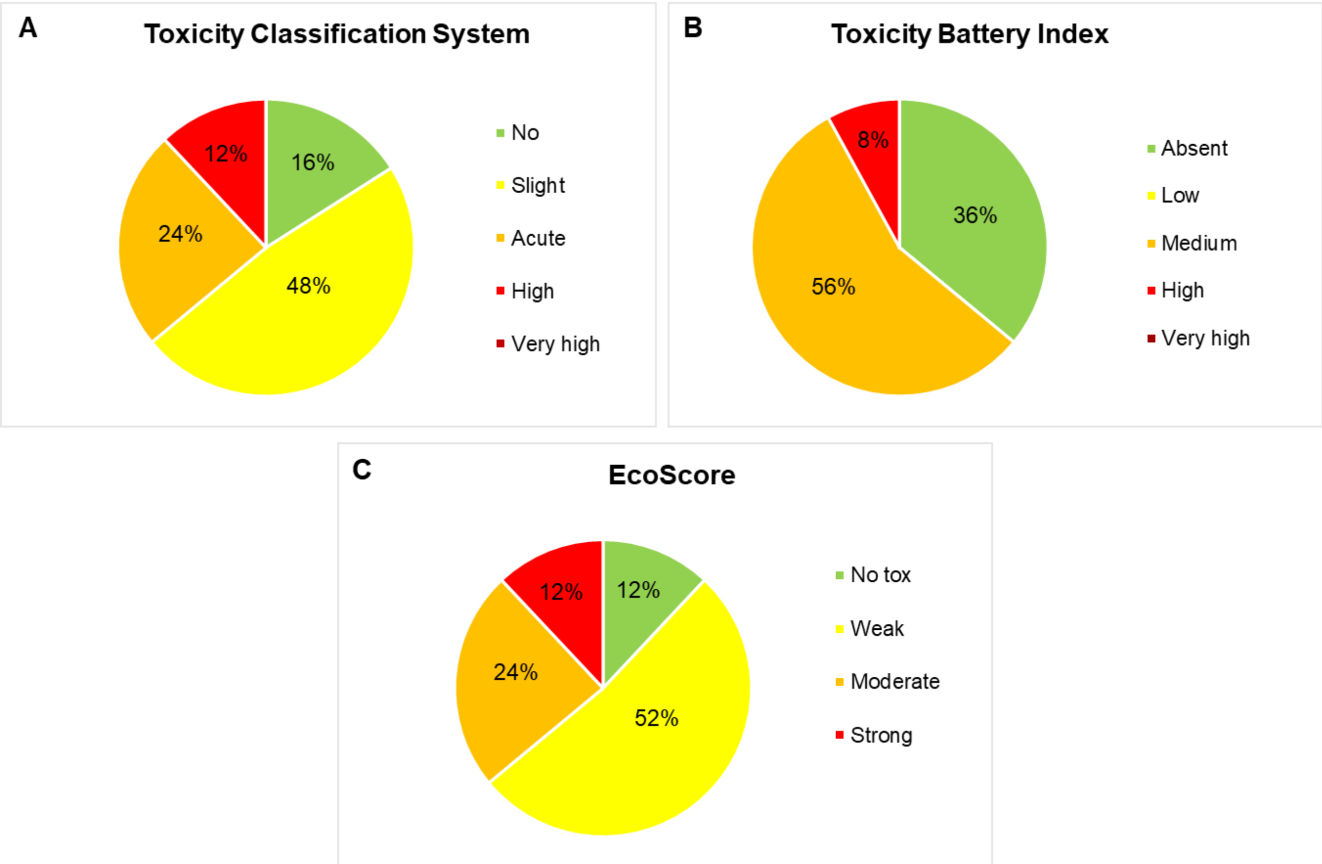

Figure 10 - Classes distribution of the undiluted samples (nominal concentration 100 g/L) according to the Toxicity Classification System (TCS - Persoone et al. 2003), the Toxicity test Battery integrated Index (TBI - Manzo et al. 2014) and the EcoScore System (EC - Lors et al. 2018).

Diluted samples were classified according to two indexes (TCS and ES), because the third system (TBI) was not applicable (Table 13). Three samples (#34, 35, 39) were confirmed not toxic by the different systems applied, as well as the two higher toxic samples (#6 and 11). In this case, the TCS classified many samples in the extreme categories ("No acute toxicity" and "High acute toxicity"), while the ES raked most of the samples in the intermediate classes ("Weak" and "Moderate").

Table 13 - Ranking of the diluted samples according to the Toxicity Classification System (TCS - Persoone et al. 2003), and the EcoScore system (EC - Lors et al. 2018).

Toxicity Classification System				
Sample #	**Hazard Class**	**Class weight score %**	**Toxicity**	
12	I	-	No acute	☺
34	I	-	No acute	☺
35	I	-	No acute	☺
39	I	-	No acute	☺
30	I	33.3	No acute	☺
3	II	33.3	Slight acute	☹
9	II	33.3	Slight acute	☹
16	II	33.3	Slight acute	☹
17	II	33.3	Slight acute	☹
24	II	33.3	Slight acute	☹
27	II	33.3	Slight acute	☹
28	II	33.3	Slight acute	☹
32	II	33.3	Slight acute	☹
26	II	33.3	Slight acute	☹
31	II	66.7	Slight acute	☹
1	III	33.3	Acute	☠
7	III	33.3	Acute	☠
29	III	33.3	Acute	☠
15	III	50.0	Acute	☠
18	III	66.7	Acute	☠
4	III	83.3	Acute	☠
10	IV	55.6	High acute	☠☠
14	IV	66.7	High acute	☠☠
6	IV	77.8	High acute	☠☠
11	IV	77.8	High acute	☠☠

EcoScore System		
Sample #	**EcoScore**	**Intensity of toxicity**
34	0	No tox
35	0	No tox
39	0	No tox
12	6	Weak
28	8	Weak
17	11	Weak
24	14	Weak
9	17	Weak
32	17	Weak
29	20	Weak
3	22	Weak
16	22	Weak
31	22	Weak
27	22	Weak
30	22	Weak
1	33	Weak
26	39	Moderate
15	42	Moderate
18	44	Moderate
7	56	Moderate
10	64	Moderate
14	67	Moderate
4	67	Moderate
11	92	Strong
6	95	Strong

Regarding the classification of the undiluted samples through the TCS (Figure 11A), 5 samples (20%) were non-hazardous, 10 samples (40%) were slightly hazardous, 6 samples (24%) were hazardous, and 4 samples (16%) were highly hazardous. None of the samples was allocated in the last class of the system ("Very high acute hazard").

The EC system (Figure 11B) classified 3 samples (12%) as non-toxic, 13 samples (52%) as weakly toxic, 7 samples (28%) with a moderate toxicity, and 2 samples (8%) as strongly toxic.

Moreover, the TCS and ES systems demonstrated a similar ranking ability of the undiluted samples among the extreme categories. Samples 34, 35, and 39 were classified "non-toxic", as well as samples 4, 6, and 11 were defined "highly toxic" by both systems.

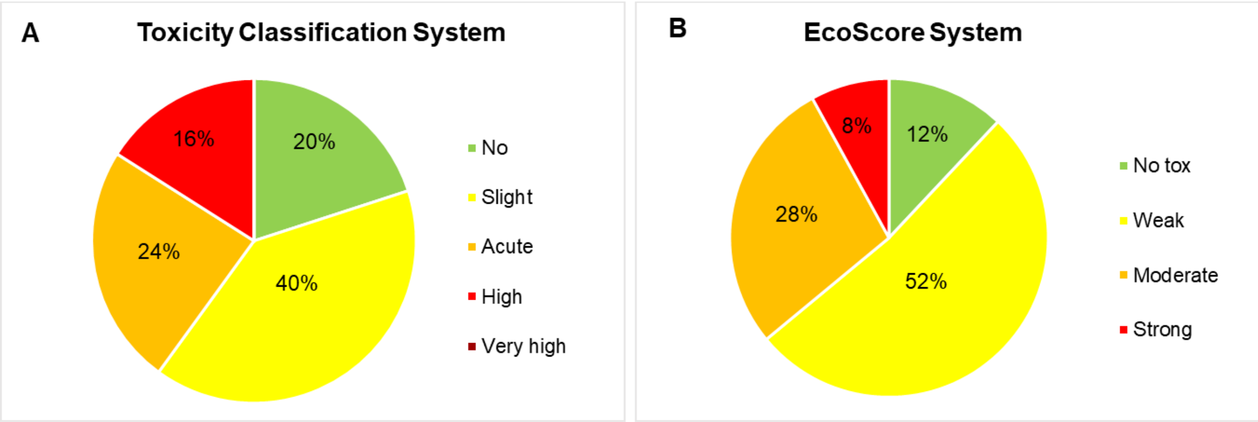

Figure 11 - Classes distribution of the diluted samples according to the Toxicity Classification System (TCS - Persoone et al. 2003), and the EcoScore System (EC - Lors et al. 2018).

Interestingly, comparing the classification of undiluted and diluted samples among the same system (Figure 12), no significant differences were observed in terms of number of samples per class, especially for the EcoScore System (Figure 12C-D). Indeed, the undiluted and diluted condition allowed the classification as "non-toxic" of the same three samples (#34, 35, 39), as weakly toxic of 12 out 13 samples, as "moderate" of 5 samples (#7, 10, 14, 18, 26), and as strongly toxic of samples 6 and 11.

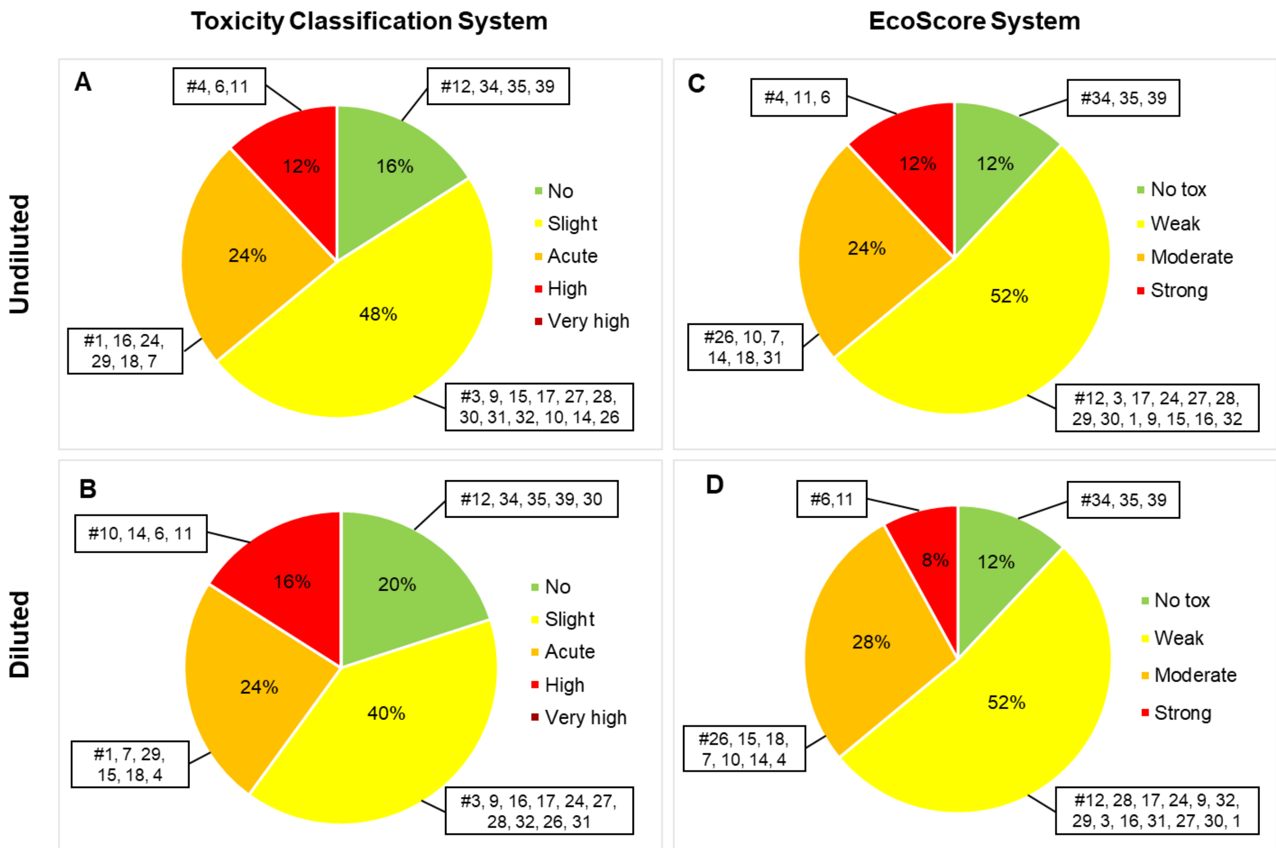

Figure 12 - Comparison between undiluted (A and B) and dilute (C and D) samples classification according to the Toxicity Classification System (TCS - Persoone et al. 2003), and the EcoScore system (EC - Lors et al. 2018).

Differences between samples with extreme biological effects

The ecotoxicological classification has clearly identified as non-toxic the samples #34, 35, 39, while as the most toxic samples 6 and 11. To find some characteristics that could explain this difference in the biological effects, the physicochemical characteristics of these samples (as both raw sands and leachates) were analysed and summarized in Table 14.

Table 14 - Summary of the characteristics of non-toxic and higher toxic samples.

Ecotox classification	Sample #	Sands						Leachates	
		Metals cast	Original sands used	Binding system				pH	EC (µS/cm)
				Green	Resin	Core	Shell		
No toxicity	34	Iron	Silica		X	Furan no-bake	Furan no-bake	5	483
	35	Iron	Silica		X	Shell molding	Shell molding	7	12.5
	39	Iron	Silica	X		Bentonite	Furan no-bake; Phenolic no-bake	7	190.5
High toxicity	6	Steel	Silica, zirconium, chromite, cerabeads		X	Sodium silicate - ester cured	Phenolic/urethane no-bake; Ashland; Shell molding	8	1756
	11	Steel	Silica, chromite		X	Sodium silicate - ester cured	Sodium silicate - ester cured; Phenolic/urethane no-bake	8	1290

Exhausted sands

All the three not-toxic samples (#34, 35, 39) derived from iron cast foundries, while the two most toxic samples (#6 and 11) came from steel cast foundries. Moreover, the two groups of samples shared similarity in the type of original sands used. The non-toxic samples derived from silica sands, while the toxic ones arose from a mixture of silica and zirconium chromite sands. Regarding the binding system, most of the selected samples (4 out 5) derived from resin binding systems, which cause the contamination of sands with organic compounds, and only one from a green one. However, even the green system involves the use of resin in the shell production/composition. This caused the contamination of all non-toxic samples by furan and phenolic no-bake binders, as well as by the resin used in the shell molding process. On the other hand, the higher toxic samples were bonded with phenolic/urethane no-bake and contaminated by the resins used in the ashland and shell molding processes but differ from the non-toxic because of the binding of core and shell with sodium silicate-ester cured.

Physical-chemical characteristics of leachates

The two groups of samples share congruity in the pH and the EC values of the obtained leachates. The non-toxic samples had neutral/acid pH (5-7) and low/medium EC (12.5-483), while the toxic samples had the same basic pH (8) and the highest EC values, well above 1000 µS/cm.

Chemical characteristics of leachates

Among the chemical analyses on leachates performed for the recovery or for landfill disposal of sands, no information was useful to identify some traits that could explain the differences between the two groups of samples. According to the analysis carried out on leachates by the producer for the recovery of non-hazardous waste (MD 186/2006) (Table 5), sample 11, as well as 34 and 39, were strongly below the limit values provided by of the Italian legislation, with many parameters even below the quantification limit of the analyses. The only exception was the COD value of the sample 39 (37.7 mg/L), that exceeded the legislation limit of 30 mg/L. Giving the analysis carried out on leachates by the producer to allow the landfill disposal of the sands (MD 27/09/2010) (Table 6), sample 6, as well as 34 and 39, were strongly below the limit values, with many parameters even below the quantification limit of the analyses. The only exception was the DOC value of the sample 34 (143 mg/L), that exceeded the legislation limit of 100 mg/L.

Conclusion

Most of the analysed samples (>60%) were ranked between non-toxic and slight toxic class by the different classification system applied, with three of them (12%) clearly classified as non-toxic, while a quarter of the samples had an intermediate toxicity. A minority (8-16%) were recognized as highly toxic, with two of them (8%) clearly recognized as the higher toxic.

The differences between non-toxic and variously toxic samples are not easily attributable, because of high variability of the samples, mainly due to the different industrial processes that generated them. However, some common aspects were identified among the two extreme classes (non-toxic and highly toxic) (Figure 13).

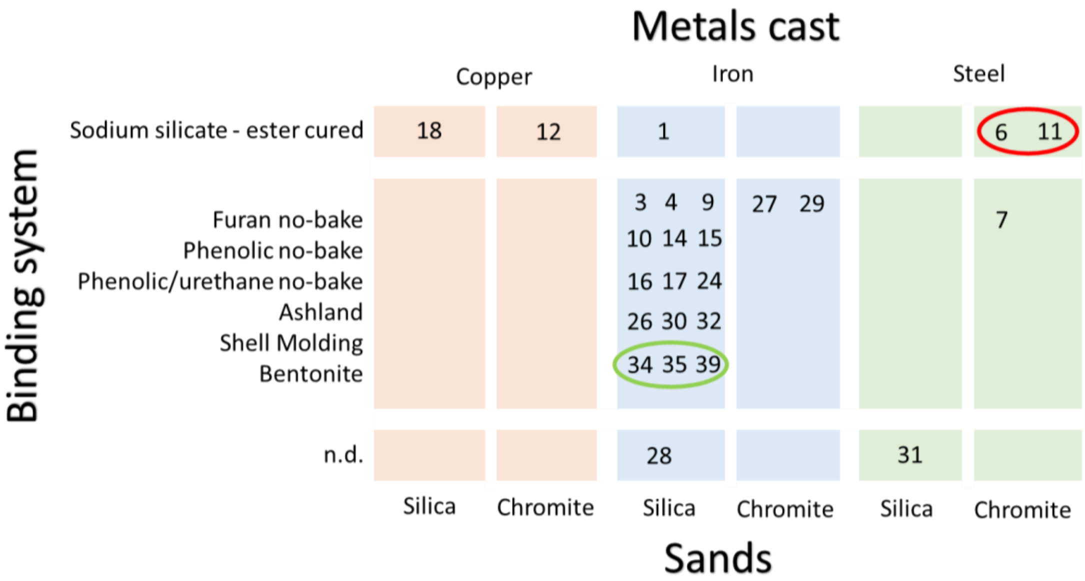

Figure 13 - Distribution of sand samples according to the type of metal cast, binding systems and original sands used. Green circle highlights the non-toxic samples, red circle the highly toxic ones.

The higher toxic samples differed from the non-toxic ones by the cast metal (steel vs iron), the presence of chromite sand, and the sodium silicate-ester cured binder. Moreover, the derived leachates were characterized by the alkaline pH and the higher electric conductivity values. Even if these data were not useful for a definitive attribution of the toxicity causes, they could be clues for starting further investigations.

From an ecotoxicological point of view, even if the diluted samples analysis could be more accurate, the substantially similarity of ranking between undiluted and diluted samples, might suggests the possibility of the application of the sole undiluted samples analysis. That could allow a less expensive and time-saving screening of the ecotoxicological traits of the waste materials.

References

EN 12457-2. 2002. “Characterisation of Waste - Leaching - Compliance Test for Leaching of Granular Waste Materials and Sludges - Part 2: One Stage Batch Test at a Liquid to Solid Ratio of 10 l/Kg for Materials with Particle Size below 4 Mm (without or with Size Reduction).”

EN ISO 11348-3. 2018. “Water Quality - Determination of the Inhibitory Effect of Water Samples on the Light Emission of Vibrio Fischeri (Luminescent Bacteria Test) - Part 3: Method Using Freeze-Dried Bacteria.”

Lors, Christine, Jean François Ponge, and Denis Damidot. 2018. “Environmental Hazard Assessment by the Ecoscore System to Discriminate PAH-Polluted Soils.” *Environmental Science and Pollution Research* 25 (27): 26747–56. https://doi.org/10.1007/s11356-017-9906-4.

Manzo, Sonia, Simona Schiavo, Pellumb Aleksi, and Afrim Tabaku. 2014. “Application of a Toxicity Test Battery Integrated Index for a First Screening of the Ecotoxicological Threat Posed by Ports and Harbors in the Southern Adriatic Sea (Italy).” *Environmental Monitoring and Assessment* 186 (11): 7127–39. https://doi.org/10.1007/s10661-014-3915-2.

Ministerial Decree. 2010. *“Definizione Dei Criteri Di Ammissibilità Dei Rifiuti in Discarica, in Sostituzione Di Quelli Contenuti Nel Decreto Del Ministro Dell’ambiente e Della Tutela Del Territorio 3 Agosto 2005.”*

Ministerial Decree n. 186. 2006. *“Regolamento Recante Modifiche Al Decreto Ministeriale 5 Febbraio 1998 Individuazione Dei Rifiuti Non Pericolosi Sottoposti Alle Procedure Semplificate Di Recupero, Ai Sensi Degli Articoli 31 e 33 Del Decreto Legislativo 5 Febbraio 1997, n.22.”*

Persoone, Guido, Blahoslav Marsalek, Irina Blinova, Andrea Törökne, Dzidra Zarina, Levonas Manusadzianas, Grzegorz Nalecz-Jawecki, et al. 2003. “A Practical and User-Friendly Toxicity Classification System with Microbiotests for Natural Waters and Wastewaters.” *Environmental Toxicology* 18 (6): 395–402. https://doi.org/10.1002/tox.10141.

UNI EN ISO 6341. 2013. “Determination of the Inhibition of the Mobility of Daphnia Magna Straus (Cladocera, Crustacea).”

UNI EN ISO 8692. 2012. “Water Quality — Fresh Water Algal Growth Inhibition Test with Unicellular Green Algae.”