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Ecotoxicological assessment of waste foundry sands and the application of different classification systems

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Abstract

The application of a battery of bioassays is widely recognized as a useful tool for assessing environmental hazard samples. However, the integration of different toxicity data is a key aspect of this assessment and remains a challenge. The evaluation of industrial waste leachates did not initially undergo any of the proposed integration procedures. This research addressed this knowledge gap. Twenty-five samples of waste foundry sands were subjected to a leaching test (UNI EN 12457-2) to evaluate waste recovery and landfill disposal. The leachates were evaluated using a battery of standardized toxicity bioassays composed of *Aliivibrio fischeri* (EN ISO 11348-3), *Daphnia magna* (UNI EN ISO 6341), and *Pseudokirchneriella subcapitata* (UNI EN ISO 8692), both undiluted and diluted. *Daphnia magna* and *P. subcapitata* were the most affected organisms, with significant effects caused by 68% and 64% of undiluted samples, respectively. The dilution of samples facilitates the calculation of EC50 values, which ranged from greater than the highest concentration tested to 2.5 g/L for *P. subcapitata*. The data on single-organism toxicity were integrated using three methods: the Toxicity Classification System, the toxicity test battery integrated index, and the EcoScore system. The three classifications were strongly similar. According to all applied systems, three samples were clearly nontoxic (from iron casting plants) and two were highly toxic (from steel casting plants). Moreover, the similar ranking between undiluted and diluted leachates suggests the possibility of using only undiluted leachates for a more cost-effective and time-efficient screening of waste materials. The findings of this study highlight the usefulness of integrating ecotoxicological waste assessment. *Integr Environ Assess Manag* 2024;20:2294–2311. © 2024 The Author(s). *Integrated Environmental Assessment and Management* published by Wiley Periodicals LLC on behalf of Society of Environmental Toxicology & Chemistry (SETAC).

KEYWORDS: *Aliivibrio fischeri*; Bioassay; *Daphnia magna*; Ecotoxicity; *Pseudokirchneriella subcapitata*

INTRODUCTION

Alongside, and in some cases more than, chemical analysis, bioassays are recognized as fundamental tools for environmental and health hazard assessment and management of substances and many types of complex matrixes, such as solid waste, wastewater, soil, surface water, groundwater, and personal-care products (Lopes et al., 2021; Viegas, 2021; Vita et al., 2018). Several bioassay methods have been standardized and required at the regulatory level. An example is the European Regulation on the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH; European Commission, 2006), which requires a wide range of toxicity assessments for all chemicals produced or imported in quantities exceeding 1 ton per year.

It is widely recognized that a single bioassay cannot fully describe the environmental impact of a compound, particularly for complex mixtures. To obtain the most reliable assessment of a sample's potential hazard, an appropriate battery of bioassays should be calibrated to the appropriate environmental compartment (Xu et al., 2020). Bioassay batteries have been used to describe the ecotoxicity of a wide variety of mixtures, matrixes, and treatments, such as contaminated drinking water (Alias, Feretti, et al., 2022), degraded soil amendment (Alvarenga et al., 2018), organic waste (Huguier et al., 2015), wastewater (Babić et al., 2019; Bedoui et al., 2015; Bertanza et al., 2021; Laquaz et al., 2018), leachates from industrial and construction materials (Alias et al., 2021, 2023), and waste from livestock manure (Delgado et al., 2013; Heisterkamp et al., 2019).

The European Union has identified 15 hazardous properties (HP) characteristic of waste materials (European Commission, 2018). The ecotoxicological property was assigned

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the number 14 and is therefore designated as HP14. Although the ecotoxicological assessment of waste is a legal requirement in Europe for HP14 evaluation, the methods have not yet been validated. Several different batteries have been proposed over the decades to classify the waste according to its impact on the environment. *Aliivibrio fischeri* and *Cerodaphnia dubia* were the most cost-effective batteries among the six tests (Pandard et al., 2006). Pablos et al. (2009) proposed a battery of tests including toxicity tests on *D. magna* and *X. laevis*, an in vitro test for defense and viability of the fish cell line RTG-2, and the DR-CALUXs assay for detecting dioxin-like compounds. Recently, Hennebert (2018) recommended the use of a highly standardized battery comprising three aquatic organisms: *fischeri*, *Pseudokirchneriella subcapitata* or *Desmodesmus subspicatus*, and *D. magna*, and four terrestrial organisms, *Arthrobacter globiformis*, *Brassica napus*, *Avena sativa*, *Eisenia andrei*, and *Eisenia fetida*, together with the corresponding concentration limits (the maximum concentration) of a waste leachate that is sufficient to classify the waste as environmentally hazardous. In addition to waste characterization, *A. fischeri*, *P. subcapitata*, and *D. magna* are recognized as a valuable battery of aquatic organisms for assessing water quality (van der Oost et al., 2017) and the impact of environmental contaminants of emerging concern, such as drugs (Lomba et al., 2020), by-products of textile dyeing industries (Methneni et al., 2021), and pharmaceuticals and personal-care products (Wang et al., 2021). The three organisms in question serve as bioindicators representing the three trophic levels (producers, primary, and secondary consumers). Furthermore, they demonstrate differential sensitivity and allow testing of acute and subacute toxicity in whole organisms. Due to these characteristics, they are considered reliable representations of organisms living in the aquatic compartment of the ecosystem.

The strength of this toxicological approach can be attributed mainly to its standardization. Indeed, the test procedures for each of these organisms are standardized internationally by the International Organization for Standardization (ISO) and the Organisation for Economic Cooperation and Development. These improvements make these tools robust for toxicological assessment. In addition, because many bioassays are available in a form that does not require continuous cultivation of organisms, known as "microbiotests," their ease of performance has proven useful for routine use (Mankiewicz-Boczek et al., 2008).

Integrating toxicity data from different endpoints and organisms is a key issue and remains a challenge. Several classification systems have been proposed in the literature to assess the toxicological risk of chemicals or mixtures. Madia et al. (2021) suggested the integration of different toxicological data to assess the carcinogenicity of chemicals. In the environmental field, many classifications have been proposed to assess the toxicity of sediments and watershed water (Hartwell, 1997; Wei et al., 2008), natural water and wastewater (Bertanza et al., 2021; Persoone et al., 2003), river sediment (Ahlf & Heise, 2005), marine sediment (Losso et al., 2007; Manzo et al., 2014; Prato et al., 2012), and polluted soil (Foucault et al., 2013; Kim et al., 2022; Lors et al., 2018). Three of these systems were used in this study. The Toxicity Classification System (TCS) proposed by Persoone et al. (2003) was developed to classify the toxicity of water or wastewater, industrial effluent, and soil and landfill leachates. The system is based on two parameters: an acute hazard/toxicity index divided into five classes (from "not acutely hazardous/toxic" to "highly acute hazardous/toxic") and a weight score for each class to indicate the quantitative importance of the effects. The "toxicity test battery integrated index," described by the Italian Institute for Environmental Protection and Research (ISPRA, 2011), has been proposed for screening the ecotoxicological risks of sediment elutriates, porewater, and sediment suspensions in different marine ports. This system calculates the "ecotoxicological risk" by weighting the type of endpoint observed, the type of environmental matrix analyzed, and the degree of agreement among the test results. The EcoScore system (EC), defined by Lors et al. (2018), was used to assess the environmental hazard of PAH-contaminated soils. The system classifies the samples into four levels of toxicity.

These methodologies were applied to a set of waste foundry sands (WFS), which are a specific type of industrial waste produced by the casting industry. A key step in casting is mold making. This process requires clean, uniformly sized, high-quality silica sand, which is bonded into molds by two main molding processes: clay-bonded or chemically bonded. Clay-bonded sands, also known as green sands, contain silica sand (85%–95%), bentonite clay (4%–10%), bituminous coal (2%–10%), and water (2%–5%), whereas chemically bonded sands or resin sands contain silica sand (93%–99%) and organic resin binder (1%–3%; Zhang et al., 2014). During the casting process, the sand is recycled several times to reduce the use of virgin sand (Tittarelli et al., 2018). The primary steps in reconditioning sand include screening, metal removal, and sieving to remove fines and oversized agglomerates. The residual binder is removed through a variety of techniques, including mechanical treatment (e.g., friction, impact, pneumatic chafing, etc.), thermal treatment, and wet scrubbing (JRC, 2022). During mechanical reclamation, the sand is crushed to achieve the desired grain size. Dry abrasion is then used to separate the binder from the sand grains. Thermal reclamation entails the precrushing of the sand, followed by the combustion of all organic binders and carbonaceous additives (USEPA, 2014). After several rounds of reconditioning, the properties of the sands are lost, rendering them unsuitable for the casting processes. Consequently, the sand particles are discarded and become WFS. The worldwide foundry sector produces more than 100 million tons of WFS annually (Ahmad et al., 2022). Approximately 3000 foundries in Europe generate 6 million tons of this amount (Delgado & Garitaonandia, 2017). Waste foundry sands are a useful resource, although concerns have been raised regarding the potential for the release of dangerous compounds during their storage or disposal (Rayjadhav & Shinde, 2021). Furthermore, the handling of WFS has been linked to

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the generation of high levels of emissions that enter the working environment (Mitterpach et al., 2017). Once they have left the foundry, the recovery of WFS depends on their chemical characteristics, which are highly variable (Cioli et al., 2022). The potential applications of WFS are several, ranging from those in the construction sector (replacement of fine aggregates in concrete, cement plants, and structural fillings in road construction; Ahmad et al., 2022; Vinoth et al., 2022) or as covering material in landfills (USEPA, 2014) to the most recent in the ceramics and glass sectors (Savić et al., 2021; Silva et al., 2020). In particular, unbound applications of WFS may result in direct contact between the material and water and soil matrixes, thereby increasing the risk of contamination of living organisms in the environment.

The legislative requirements for the reuse of WFS are quite distinct, and there is a paucity of well-defined management strategies. In numerous countries, including the United States (USEPA, 2014), France (CEREMA, 2019), and Italy (Ministerial Decree n. 186, 2006), the reuse of WFS is associated with the compliance of parameters in the eluate obtained from leaching tests and regulatory standards.

Few studies have evaluated the environmental toxicity of WFS, which is strongly related to the metal and organic contaminant content (Zhang et al., 2014). Zhang et al. (2013) described the toxic effects of WFS leachates on the luminescent bacteria *Vibrio fischeri*. Curieses et al. (2016) evaluated the cyto/genotoxic effects of both leachates and solid foundry sands on the earthworm *E. fetida*. Mastella et al. (2014) evaluated the toxicity of concrete containing WFS using a battery composed of *D. magna*, *Allium cepa*, and *E. fetida*, which describes the effects produced on each organism.

To the best of our knowledge, this is the first study aimed at the ecotoxicological characterization of many WFS leachates using a standard battery of bioassays based on the bacteria *A. fischeri*, the crustacean *D. magna*, and the algae *P. subcapitata*. In addition, three classification systems for integrating toxicity data were applied and compared. Figure 1 summarizes the study design.

MATERIALS AND METHODS

Foundry plants and sand samples

Twenty-five foundry plants in northern Italy were included in this study. The plants operated in the iron, steel, and copper casting processes. Samples of WFS, derived from several reuse cycles of sand before the final disposal, were collected from May 2021 to October 2021. Together with WFS sampling, an ad hoc questionnaire was sent to the representatives of the plants to collect information on the casting processes, including details on the type and origin of the virgin sands used for the molding, the binder system, and the chemical characteristics of the leachates of the waste sands. These analyses were routinely done by the foundries according to the Italian legislation for the recovery of nonhazardous waste (Ministerial Decree n. 186, 2006).

Leaching tests

Leaching tests were performed on the samples according to the European regulation for the characterization of waste (EN 12457-2, 2002) and adopted in Italy for the evaluation of recovery (Ministerial Decree n. 186, 2006) or landfill disposal (Legislative Decree n. 121, 2020). The tests were performed by mixing the homogenized samples with

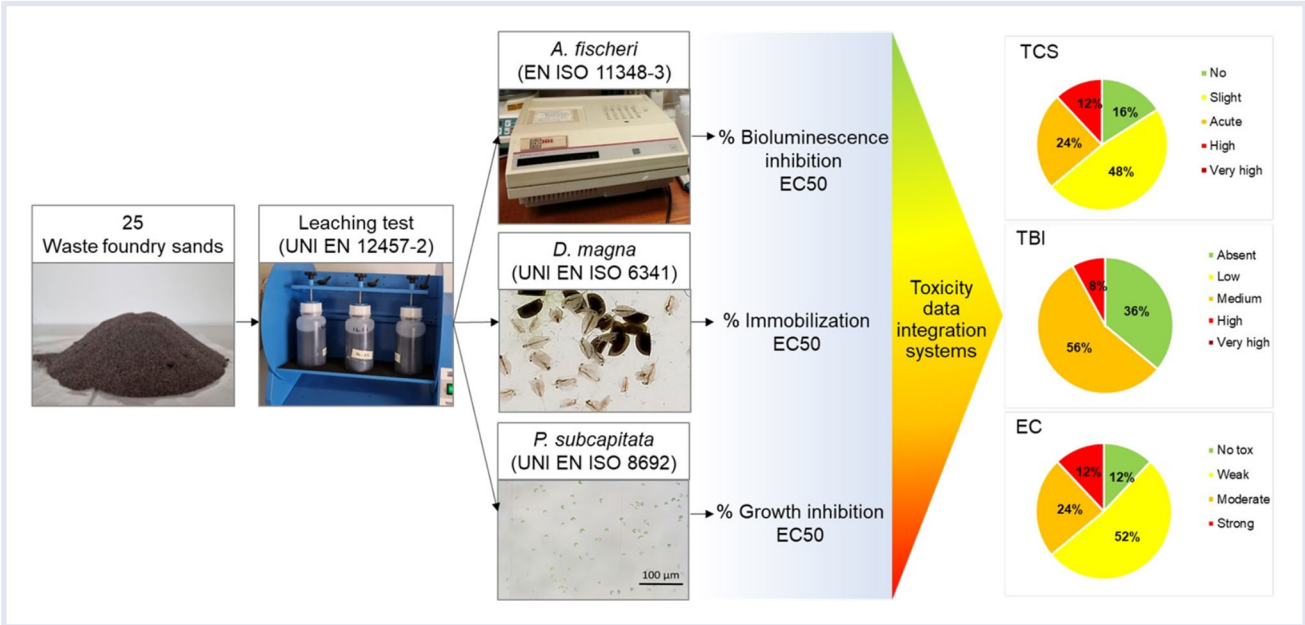


FIGURE 1 Schematic representation of the study design. EC, EcoScore system; TBI, Toxicity test Battery integrated Index; TCS, Toxicity Classification System

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demineralized water (pH7) at a liquid-to-solid ratio of 10 L/kg to obtain eluates with a nominal concentration of 100 g/L (the nominal concentration is equivalent to the extracted compounds of originally 100 g sample/L); these eluates were known as undiluted leachates. Moreover, to study the toxicity, four eluates with different sample concentrations were obtained by performing additional leaching tests at higher liquid-to-solid ratios (20, 40, 60, and 120 L/kg), obtaining leachates with nominal concentrations of 50, 25, 12.5, and 6.25 g/L, respectively. For all leaching tests, the mixtures (sample and leaching solution) were placed on a tightly closed rotary shaker (VELP) and agitated for 24 h, rotating at 10 ± 2 rpm. The solutions were filtered through 0.45 μm filters. Electrical conductivity (σ) and pH were measured with a multiparameter instrument equipped with pH and conductivity probes (Hanna Instruments). The leachates were then stored at 4 °C.

Aliivibrio fischeri bioluminescence inhibition assay

Toxicity to the bioluminescent bacteria *A. fischeri* was measured using the Microtox Toxicity Test according to the standard procedure (EN ISO 11348-3, 2018). As a first step, the 81.9% screening test was performed on undiluted leachates. Subsequently, samples exhibiting an effect greater than 30% (arbitrary cutoff of toxicity) were further tested for EC50 by serial dilutions at nominal concentrations of 50, 25, 12.5, and 6.25 g/L. Microtox diluent (2% NaCl) was used as a negative control. The decrease in luminescence was evaluated after 5, 15, and 30 min of exposure using a Microbics Model 500 Toxicity Analyzer according to the manufacturer's instructions (Microbics Corporation). The results were expressed as the percentage of bioluminescence inhibition relative to the control and, when possible, as the half maximal effective concentration (EC50) calculated by probit regression with a confidence interval (CI) of 95%.

Daphnia magna acute immobilization test

The assay was performed by using Daphtoxkits F (Ecotox LDS), according to the standard procedure (UNI EN ISO 6341, 2013). As a first step, leachates were evaluated at a concentration of 100 g/L without any modification (pH or salts). Samples having an effect greater than 10% (arbitrary cutoff of toxicity) were further tested for EC50 by serial dilutions at nominal concentrations of 50, 25, 12.5, and 6.25 g/L. In all, 20 neonates of *D. magna* (<24-h-old) were used for each test group, divided into four groups of five animals, each group in 10 mL of test medium. Standard freshwater was used as the negative control. Effects on crustacean movement or death were determined by visual inspection at 24 and 48 h (animals unable to swim within 15 s after gentle agitation of the test container were considered immobile). The percentage of immobilized animals was determined, and the EC50 was calculated by probit regression with a 95% CI.

Pseudokirchneriella subcapitata growth inhibition test

The *P. subcapitata* growth inhibition assay was performed according to a standard procedure (UNI EN ISO 8692, 2012) using Algaltoxkit F (Ecotox LDS). A miniscale test procedure was used. The initial algal density was 10^4 cells/mL in 2 mL of each sample at a concentration of 100 g/L, adjusted to culture conditions using concentrated nutrient solutions. Samples were incubated for 72 h with orbital shaking at 23 ± 1 °C and at 10 000 lux. Samples exhibiting effects greater than 30% (arbitrary cutoff of toxicity) were further tested for EC50 by serial dilution at nominal concentrations of 50, 25, 12.5, and 6.25 g/L. The algae growth medium was used as a negative control. The test was done in quadruplicate. Algal growth rates were calculated by reading the optical density at 690 nm. Cell density was determined using a six-point standard curve (from 1×10^4–1×10^7 cells/mL). Otherwise, for colored or turbid samples, algal growth was determined by manual counting using a hemocytometer. The assay was considered valid if the average growth rate in the control was at least 1.4/day and the coefficient of variation of the growth rate in the control replicates did not exceed 5%. Toxicity was expressed as the percentage of growth inhibition (*I*%), and the EC50 values were calculated by probit regression with a 95% CI.

Ecotoxicity classification systems

To comprehensively assess the samples, an integrated toxicity classification approach was implemented. Three different systems were applied and compared. A detailed description of each classification system is provided in the Supporting Information. Below is a brief summary of each methodology.

Toxicity Classification System. The TCS (Persoone et al., 2003) was applied to undiluted leachates using two parameters: the acute hazard class and weight score for each class, which indicated the quantitative significance of the effects. Full-dilution series assays allowed the calculation of L(E)C50 values and the derived toxic units (TU) using the formula: $TU = [1/L(E)C50] \times 100$. Two parameters were assigned: an acute toxicity class and a weight score for each class, which indicated the quantitative significance of the effects. The toxicity scale was used to classify the samples according to the intensity of acute hazard or acute toxicity. These levels were also highlighted by color coding from green to dark red (Supporting Information Table 1S).

Toxicity test Battery integrated Index. The toxicity test Battery integrated Index (TBI; ISPRA, 2011; Manzo et al., 2014) classified the undiluted leachates into ecotoxicological risk classes (from "absent" to "very high"). The toxicity scale was used to classify the samples according to their ecotoxicological risk level. These levels were also highlighted by color coding from green to dark red (Supporting Information Table 2S).

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EcoScore system. The EcoScore system (EC; Lors et al., 2018) is based on scores calculated by assigning a score between 0 and 100 to each endpoint of the battery as a function of its intensity. The toxicity scale was used to classify the samples according to the intensity of toxicity. Scores were also highlighted using color coding from green to red (Supporting Information Table 3S).

RESULTS

Characteristics of WFS

The data collected from the questionnaires revealed that 76% of the foundries produce castings in cast iron, 16% in steel, and 8% in copper alloys. The main type of virgin sand used is silica sand, which comes from France, Italy, and Portugal. Eight foundries used the green molding process, nine used the resin molding processs, and five used both. For green molding, bentonite and mineral black are used to activate the sand to obtain the molds, whereas for resin molding, phenolic, furan, or isocyanate agglomerates or additives are used. The cores, for which only resin molding is required, are obtained by different processes depending on the aggregates or additives and the catalysts added (Table 1). Among the foundries from which the tested sands come, 17 applied only mechanical treatments for the regeneration of the sand, and six foundries used both mechanical and thermal treatments. These data were not available for two foundries. The dry weight content of each sample was also determined, ranging from 85.4% to 99.9% (Supporting Information Table 4S).

Characteristics of foundry sand leachates

Among the information provided by the plants, routine chemical analyses of the leachates, done according to the Italian regulations on waste recovery, were collected (Table 2). Most of the parameters were within legislative limits. However, some other parameters exceeded the limit values. Among these parameters, four were frequently exceeded: fluorides, copper, nickel, and chemical oxygen demand (COD; Figure 2). In particular, resin-molded WFS release more copper, nickel, and COD than WFS produced by green processes.

Leachates were obtained from 25 samples of WFS. The nominal concentration of the undiluted leachates was 100 g/L. Fourteen of them (56% of the total) were colored, ranging from a slight tint to a darker color (pictures of all leachates are shown in Supporting Information Figure 1S). The pH had a median value of 7.0, ranging from 6.0 to 9.5, with a single outlier of pH = 5. Electrical conductivity values were highly variable, ranging from 12.5 to 717 µS/cm (median value = 415.0 µS/cm), with three outliers of higher conductivity (886, 1290, and 1756 µS/cm). Detailed data on the pH and electrical conductivity (σ) values of the leachates are provided in Supporting Information Figure 2S and Table 5S. It is also important to note that almost half of the leachates (12/25) appeared thickened, despite the filtration step after the leaching test.

Assessment of leachate biological effects

The solutions obtained from the leaching tests of 25 WFS were tested using the standard ecotoxicity battery for the aquatic compartment consisting of *A. fischeri*, *D. magna*, and *P. subcapitata* under undiluted and diluted conditions.

Undiluted WFS leachates. The *A. fischeri* screening test revealed that eight samples had no effect on bacterial bioluminescence (32%), and 12 samples (48%) caused slight inhibition of bioluminescence (<30% inhibition after 30 min). Finally, five samples (20%) exhibited strong or complete inhibition of bioluminescence (Figure 3A). The *D. magna* immobilization test revealed that most samples (17/25, 68%) did not alter the mobility behavior of the animals (≤10% of immobilization after 48 h). The remaining eight leachates (32%) caused between 10% and 100% immobilization of *D. magna* (Figure 3B). Some of the thickened WFS leachate (samples 5, 7, 10) formed a kind of film around the daphnids, covering them and making them visible only by active search. Slow movements of the filtering limbs of the crustaceans indicated that they were alive but unable to actively move in the test vessel, suggesting a phenomenon of physical toxicity. Regarding the effect on *P. subcapitata*, two samples (samples 24 and 25; 8%) did not inhibit algal growth, and seven samples (28%) caused a slight inhibition of growth (<30% inhibition after 72 h). Most samples (64%) were able to inhibit proliferation above 30% (Figure 3C). The same phenomenon of physical toxicity described for *D. magna* was induced by leachate samples 4, 5, 6, 7, 8, 10, 12, 14, 15,16, 19, 20, and 22, on *P. subcapitata* cells, whose growth was strongly affected by the dense matrix formed. Examples of algae cultures in undiluted leachates are shown in Supporting Information Figure 3S.

Diluted WFS leachates. Table 3 summarizes the EC50 values of the diluted samples from the three ecotoxicity tests. Five samples (20%) were diluted for the *A. fischeri* toxicity test. Samples 4 and 8 confirmed the highest toxicity with the lowest EC50 values (<5 and 6.6 g/L, respectively), whereas sample 1 had an intermediate EC50 value (31.6 g/L). Samples 14 and 16 displayed low toxicity with EC50 values around the maximum tested concentration (95.4 and 97.7 g/L, respectively). Eight samples (32%) were diluted for the *D. magna* toxicity test. For two samples (11 and 21), the EC50 values were greater than the highest concentration tested (100 g/L). Samples 3, 4, and 8 displayed intermediate toxicity, with EC50 values of 57.0, 29.6, and 44.7 g/L, whereas samples 5, 7, and 10 were the most toxic, with the lowest EC50 values (17.7, 13.3, and 5.6 g/L, respectively). Twenty samples (80%) were diluted to obtain the *P. subcapitata* toxicity test. Half of the samples (13/25) had EC50 values greater than the highest tested concentration (100 g/L). Five samples (20%) displayed an intermediate EC50 between 89.1 and 31.6 g/L. Two samples (7 and 10) exhibited the highest toxicity with EC50 values of 8.4 and 2.5 g/L, respectively.

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TABLE 1 Type of virgin sands used in the foundries studied and characteristics of the waste foundry sand samples

Sample #	Metals cast	Type of virgin sand (origin)	Type of molding		Binder system		Reclamation treatment	
			Green	Resin	Core	Shell	Mechanical	Thermal
1	Iron	Silica (Portugal, France)		✓	Sodium silicate—ester cured	Sodium silicate—ester cured	✓	✓
2	Iron	Silica (Portugal)		✓	Furan no-bake	Furan no-bake	✓	✓
3	Iron	Silica (Portugal)		✓	Furan no-bake	Furan no-bake; Ashland; Shell molding	✓	
4	Steel	Silica, zirconium, chromite, cerabeads		✓	Sodium silicate—ester cured	Phenolic/urethane no-bake; Ashland; Shell molding	✓	
5	Steel	Silica, chromite	✓		Bentonite	Phenolic/urethane no-bake	✓	✓
6	Iron	Carbomix, american bentonite, silica (France)	✓		Bentonite	NU	✓	
7	Iron	Silica	✓		Bentonite	Ashland	✓	
8	Steel	Silica, chromite		✓	Sodium silicate—ester cured	Sodium silicate—ester cured; Phenolic/urethane no-bake	✓	✓
9	Copper	Silica (Portugal, France), chromite	✓	✓	Bentonite; Sodium silicate—ester cured	Phenolic no-bake; Phenolic/urethane no-bake	✓	✓
10	Iron	Silica (France, Italy)	✓	✓	Furan no-bake; Phenolic no-bake	Furan no-bake, Phenolic no-bake; Ashland; Shell molding	✓	
11	Iron	Silica (France)		✓	Furan no-bake	Furan no-bake	✓	
12	Iron	Silica (Italy)	✓		Bentonite	NU	✓	
13	Iron	Silica (Portugal)		✓	Furan no-bake	Furan no-bake	✓	
14	Copper	Silica (Francia)	✓	✓	Sodium silicate—ester cured	Furan no-bake; Ashland; Shell molding	✓	
15	Iron	Silica	✓		Bentonite	NU	✓	
16	Iron	Silica	✓		Bentonite	NU	✓	
17	Iron	Silica (Italy, France), chromite	✓	✓	Bentonite; Furan no-bake	Furan no-bake	✓	
18	Iron	ND	ND	ND	ND	ND	ND	ND
19	Iron	Silica (France), chromite	✓	✓	Bentonite	Furan no-bake; Phenolic/urethane no-bake	✓	
20	Iron	Silica (France)	✓	✓	Bentonite; Furan no-bake	NU	✓	✓
21	Steel	ND	ND	ND	ND	ND	✓	
22	Iron	Silica (Italy)	✓		NU	Bentonite; Ashland; Shell molding	✓	

(*Continued*)

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TABLE 1 (*Continued*)

Sample #	Metals cast	Type of virgin sand (origin)	Type of molding		Binder system		Reclamation treatment	
			Green	Resin	Core	Shell	Mechanical	Thermal
23	Iron	Silica		✓	Furan no-bake	Furan no-bake	✓	
24	Iron	Silica (Italy)		✓	Shell molding	Shell molding	ND	ND
25	Iron	Silica (Portugal)	✓		Bentonite	Furan no-bake; Phenolic no-bake	✓	

Abbreviations: NU, not used; ND, not declared.

Classification of WFS leachates

To facilitate a comprehensive interpretation of the resulting toxicity, the data were integrated using different toxicity scores.

Undiluted WFS leachates. The undiluted samples of WFS leachates were ranked according to the three classification systems, as summarized in Table 4. According to the TCS classification, four samples (16%) were nonhazardous (hazard Class I), 12 samples (48%) were slightly hazardous (hazard Class II), six samples (1, 5, 12, 14, 15, 19; 24%) were hazardous, and three samples (12%) were highly hazardous (hazard Class IV). None of the samples were classified in the last class of the system (hazard Class V). As a result of the TBI classification, nine samples (36%) were associated with no risk of ecotoxicological concern (TBI% < 5), 14 samples (56%) were related to a medium ecotoxicological risk (TBI indices ranging from 5.8 to 22.7), and two samples (8%) were at high risk with TBI indexes of 34.1 and 37.3. No sample was categorized as "low-risk" or "very high-risk." The EC system classified three samples (12%) as nontoxic (ES = 0), 13 samples (52%) as weakly toxic with ESs ranging from 22 to 33, six samples (24%) as moderately toxic ($39 < ES < 56$), and three samples (12%) as severely toxic, covering all available classes of the system.

Diluted WFS leachates. Diluted samples were classified only according to TCS and EC because the TBI system was not applicable (Table 5). After the TCS classification, five samples (20%) were nontoxic (toxicity Class I), 10 samples (40%) were slightly toxic (toxicity Class II), six samples (24%) were classified as acutely toxic (toxicity Class III), and four samples (16%) demonstrated high acute toxicity. None of the samples displayed a very high level of acute toxicity (toxicity Class V). The EC system classified three samples (12%) as nontoxic (ES = 0), 13 samples (52%) as slightly toxic ($6 < ES < 33$), seven samples (28%) as moderately toxic ($39 < ES < 67$), and two samples (8%) as severely toxic with ES of 92 and 95.

Relationship between toxicological characterization and physicochemical parameters of WFS

Although the classification systems differ in methodology, they all assigned some samples to the same opposite categories. Samples 23, 24, and 25 were identified as nontoxic, and samples 4 and 8 were classified as highly toxic. To find some characteristics that could explain these differences in biological effects, all samples were organized according to the characteristics of the sands of origin (Supporting Information Figure 4S), and the physicochemical characteristics of the most interesting five samples (both as virgin sands and as leachates) are summarized in Table 6. All three nontoxic samples came from iron casting foundries; the two highly toxic samples came from steel casting foundries. The type of virgin sand used differed between the two groups of samples. Nontoxic samples were obtained from silica sands,

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TABLE 2 Chemical characterization of leachates according to the Italian regulation for waste recovery (MD 186/2006)

			Green sands		Resin sands	
Parameter	MU	Limit value MD 186/2006	Range	Exceeding percentage	Range	Exceeding percentage
Ions						
Chlorides	mg/L	100	2.48–34	0	1–75.3	0
Fluorides	mg/L	1.5	<0.1–**5**	69	<0.2–**15.4**	50
Nitrates	mg/L	50	0.22–2	0	<1–29.8	0
Sulfates	mg/L	250	18–221	0	3–198	0
Metals						
Antimony	mg/L	-	<0.001–<0.0053	NP	<0.001–<0.01	NP
Arsenic	µg/L	50	0.743–11.2	0	<1–<10	0
Asbestos	mg/L	30	<–0–<10	0	<0.—003–<10	0
Barium	mg/L	1	0.002–0.8	0	<0.01–<1	0
Beryllium	µg/L	10	<0.086–<1	0	<0.5–<5	0
Cadmium	µg/L	5	<0.081–<1	0	<0.4–**<10**	8
Total chromium	µg/L	50	<0.3–22	0	<0.1–36	0
Cobalt	µg/L	250	<0.21–<100	0	<1–<100	0
Copper	mg/L	0.05	0.001–0.05	0	0.001–**<0.1**	25
Cyanide	µg/L	50	<5–<15	0	<5–<30	0
Lead	µg/L	50	<0.26–13.3	0	<1–**<100**	8
Mercury	µg/L	1	<0.031–<0.93	0	<0.1–<1	0
Molybdenum	mg/L	-	<0.001–0.143	NP	<0.001–<0.100	NP
Nickel	µg/L	10	<0.24–**245**	15	<1–**300**	25
Selenium	µg/L	10	<0.86–<5	0	<1–<10	0
Vanadium	µg/L	250	<0.4–20.1	0	<1–64	0
Zinc	mg/L	3	0.0028–0.4000	0	<0.01–2.58	0
Others						
COD	mg/L	30	10.7–**76**	54	<1–**792**	54
pH	-	5.5 < > 12	6.21–10.2	0	**3.9**–11	27
Electrical conductivity	mS/cm	-	166–699	NP	101.3–1100	NP
TDS	mg/L	-	145–2130	NP	13–1200	NP
Phenol index	mg/L	-	0.18–19.90	NP	0.077–0.1	NP

Note: The values in bold exceed (or could exceed) the limit values.
Abbreviations: COD, chemical oxygen demand; MD, Ministerial Decree; MU, measure unit; NP, not provided; TDS, total dissolved solids.

whereas toxic samples were obtained from a mixture of silica and zirconium and chromite sands. Regarding the binder system, most of these samples (four out of five; samples 4, 8, 23, 24) were derived from resin binder systems, which contaminate the sands with organic compounds. A single sample (25) was produced by a green system. However, even the green system involves the use of resin in shell production and composition. This contaminated all nontoxic 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 more toxic samples were bonded with phenolic urethane no-bake and contaminated by the resins used in the ashland and shell molding processes; they differed from the nontoxic samples in that the core and shell were bonded with sodium silicate-ester cured. Moreover, the leachates from the two sets of

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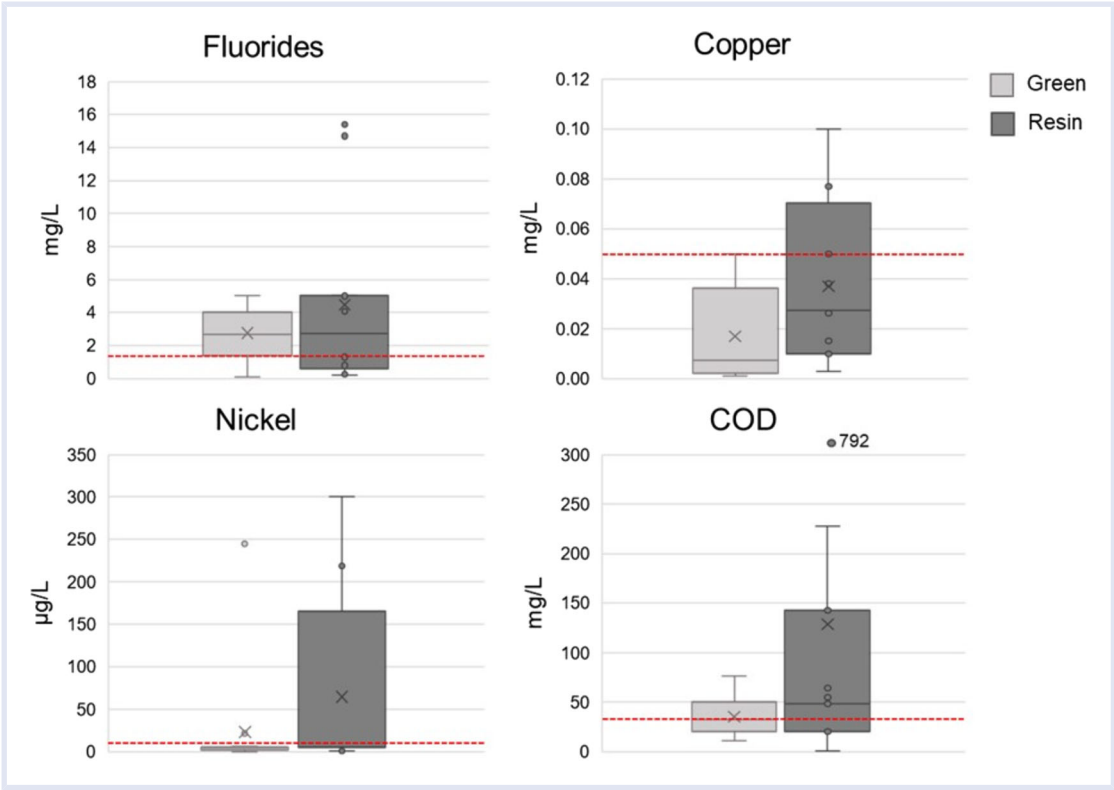


FIGURE 2 Fluorides, copper, nickel, chemical oxygen demand (COD) value distributions of green and resin sand leachates obtained according to EN 12457-2. Data are represented by box plots to emphasize the following values: minimum, 1st quartile, median, mean, 3rd quartile, maximum, and outliers. The red dotted lines represent the limit values set by the Italian legislation on waste recovery (Ministerial Decree n. 186, 2006)

samples had similar pH values and electrical conductivities. The nontoxic samples had neutral/acidic pH ($5 < pH < 7$) and low/medium electrical conductivities ($12.5 < \sigma < 483$ µS/cm), whereas the toxic samples had the same basic pH ($pH = 8$) and the highest σ values, well above 1000 µS/cm.

Although the systems demonstrated concordance among the extreme samples, this was not observed for the intermediate samples, which were classified as "slightly" or "moderate" toxic by each system. Consequently, it was not possible to unequivocally categorize these samples.

DISCUSSION

Twenty-five WFS leachates were subjected to a battery of bioassays based on living organisms, namely *A. fischeri*, *D. magna*, and *P. subcapitata*, to identify their potential ecotoxicity. The three aquatic organisms used in this study belong to different major taxonomic groups (bacteria, plants, animals) and are the highly recommended models for the correct analysis of solid waste leachates, specifically obtained according to EN 12457-2 (2002; Hennebert, 2018) because of their different sensitivity to leachable pollutants, namely polar organic compounds and metals (Weltens et al., 2014). Nearly half of the samples were found to cause negligible or low levels of toxicity, particularly to bacteria and crustaceans. On the contrary, the alga *P. subcapitata* appeared to be the most sensitive model, with most samples (64%) exhibiting greater inhibitory effects (>30% of growth inhibition) when tested in the undiluted form and the lowest EC50 value (2.5 g/L) among the diluted samples. This was often observed when *P. subcapitata* was tested against various samples, such as municipal solid waste incineration bottom ash (Ferrari et al., 1999), simulated textile and tannery wastewaters (Tigini et al., 2011), organic compounds (Li et al., 2015), and antibiotics (Li et al., 2023), likely the result of its uptake capabilities.

As individual pieces of a puzzle, the biotests were recognized as being able to discriminate between ecotoxic and nonecotoxic waste, and appropriate thresholds were proposed for each to assess HP14 Ecotoxic (Pandard & Römbke, 2013). In contrast, in this study, a data integration approach was proposed to correctly categorize samples (whether undiluted or diluted) based on three or more different results. In other words, What does the whole puzzle look like? Three classification systems for integrating toxicity data were applied and compared: TCS, TBI, and EC. All these systems have the advantage of not requiring sample dilution. However, two of them (TCS and EC) are capable of functioning even when the samples are diluted. The TCS was developed on culture-independent bioassays, whereas the others were not specifically related to such assays. Finally, although TCS and EC are based on weighted measurable effects, TBI also includes a statistical correction factor to measure the accordance level of each test result.

The systems in question are based on different parameters and have been developed and tested for the analysis of a variety of matrixes, including water or wastewater,

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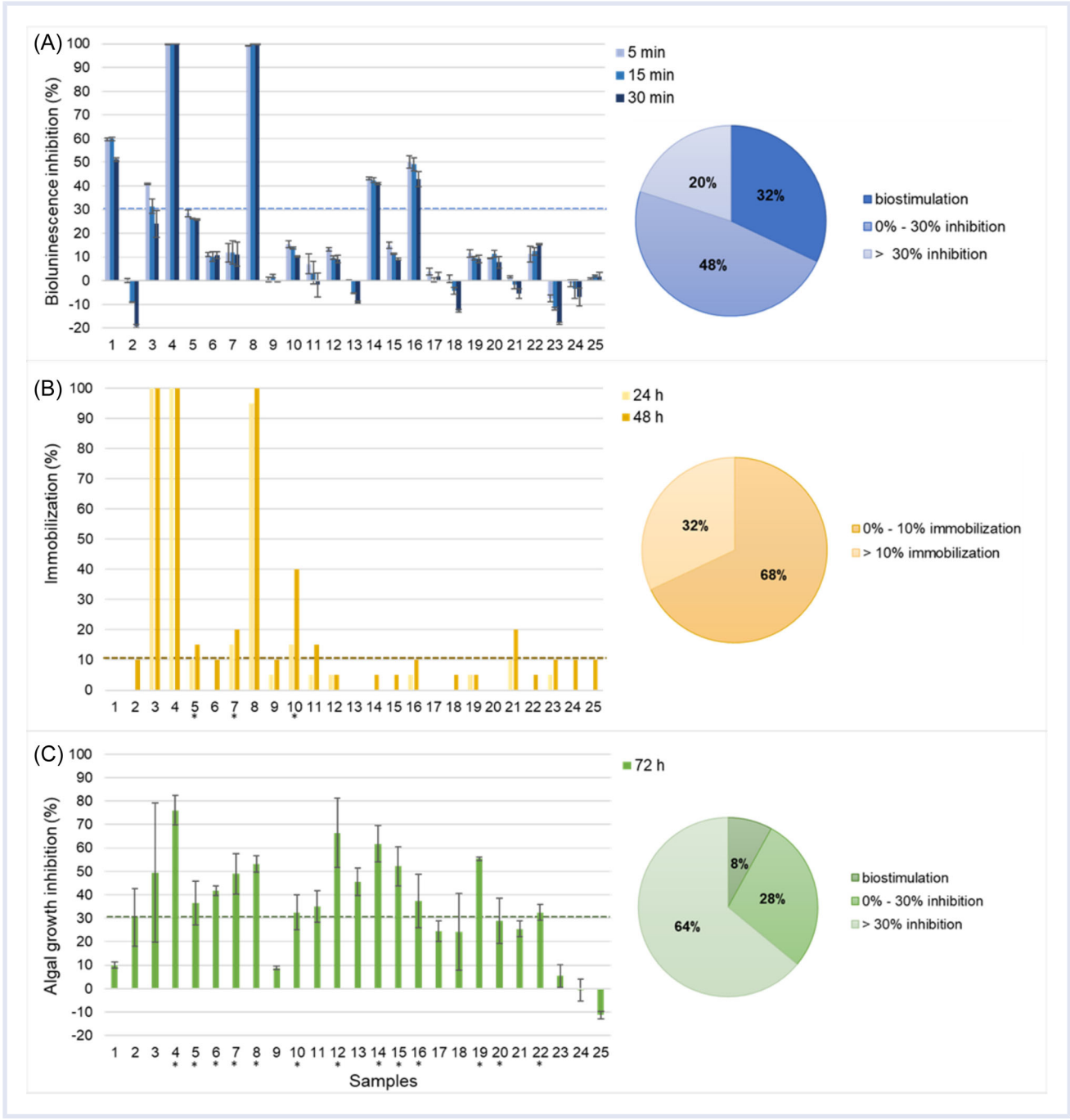


FIGURE 3 Results of the toxicity assessment of undiluted waste foundry sand leachates using (A) *Aliivibrio fischeri*, (B) *Daphnia magna*, and (C) *Pseudokirchneriella subcapitata*, expressed as a percentage of the effects (bioluminescence inhibition, immobilization, and growth inhibition, respectively; left panels) and frequency of samples according to the range of effect (right panels). The effect of inhibition was determined by comparing it with that of the corresponding negative control. Dotted lines indicate the percentage effect used as the cutoff for further analysis. The symbol (*) denotes thickened WFS leachates, which may cause physical toxicity phenomena

industrial effluents, soil, landfill leachates, and sediment. Although none of these systems had originally been used for the assessment of industrial waste leachates, all three systems demonstrated strong adaptability for the classification of WFS leachates. Most samples (>60%) were classified as nontoxic (12%–20%) and slightly toxic (40%–52%) according to the different classification systems used, and a quarter of the samples exhibited intermediate toxicity. A minority (8%–16%) were classified as highly toxic, of which two samples (4 and 8) were clearly identified as most toxic by the three systems. A third sample (3) was placed in the last class by two systems (TCS and ES) and was just above the threshold for "highly toxic" by the TBI system. It is of interest to note that the TCS and ES systems demonstrated high levels of protection, with the capacity to categorize samples into four out of five or even four out of four

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TABLE 3 Toxicity assessment of diluted waste foundry sand leachates, expressed as EC50 values and confidence intervals (95%)

	Toxicity EC50 (g/L)		
Samples #	*Aliivibrio fischeri*	*Daphnia magna*	*Pseudokirchneriella subcapitata*
1	31.6 (19.8–50.2)	NT	NT
2	NT	NT	>
3	NT	57.0 (26.8–369.9)	>
4	<5	29.6 (19.2–43.5)	31.6 (28.8–34.3)
5	NT	17.7 (13.9–22.28)	>
6	NT	NT	>
7	NT	13.3 (13.0–86.6)	8.4 (7.5–10.5)
8	6.6 (6.3–6.8)	44.7 (32.1–60.2)	33.1 (27.4–41.6)
9	NT	NT	NT
10	NT	5.6 (0.3–10.9)	2.5 (0.0–4.9)
11	NT	>	74.8 (52.4–105.3)
12	NT	NT	>
13	NT	NT	>
14	95.4 (64.5–123.1)	NT	69.0 (49.8–116.4)
15	NT	NT	>
16	97.7 (73.1–103.7)	NT	>
17	NT	NT	>
18	NT	NT	>
19	NT	NT	89.1 (76.9–106.7)
20	NT	NT	>
21	NT	>	>
22	NT	NT	>
23	NT	NT	NT
24	NT	NT	NT
25	NT	NT	NT

Abbreviations: NT, nontoxic/not tested in dilute form due to lack of toxicity; >, EC50 greater than the highest concentration tested.

available classes, respectively. On the other hand, TBI, which was less able to blend the toxicity of samples, classified them into three of the five available classes. Notably, Prato et al. (2015) reached a different conclusion. In the context of the assessment of the quality of marine sediments, TCS and TBI were compared, resulting in a high degree of concordance between the classifications provided by the two systems. The differences between nontoxic and toxic samples are not easy to explain with certainty because of the high variability of the samples, mainly resulting from the different industrial processes used to produce them. However, some common aspects were identified between the two extreme classes, and surprisingly, the type of molding (green or resin) was not one of them. The highly toxic samples differed from the nontoxic ones by the casting metal (steel vs. iron), the presence of chromite sand, and the use of a sodium silicate-ester cured binder for the core of the mold. This last aspect seems particularly interesting. Although the sodium silicate-ester cured binder has been applied widely in European nonferrous and steel foundries for its good handling properties (Carey & Sturtz, 1996), some environmental and worker health concerns arise because of the formation of formaldehyde during curing and binder decomposition when metals are poured into molds (Carey, 1995). Phenol has also been detected frequently in phenolic/ester sand leachates as a by-product generated under the influence of temperature (Bożym, 2021; Holtzer, Dańko, Zymankowska-Kumon, et al., 2016; Siddique et al., 2010). Moreover, Holtzer and collaborators (Holtzer,

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TABLE 4 Classification of the undiluted waste foundry sand leachates according to the Toxicity Classification System, the Toxicity test Battery integrated Index, and the EcoScore system (EC)

Toxicity Classification System				Toxicity test Battery integrated Index				EcoScore system		
Sample	Hazard class	Class weight score %	Hazard	Sample	TBI%	SCF	Ecotoxicological risk level	Sample	Eco-Score	Intensity of toxicity
9	I	0.0	No acute	23	−1.2		Absent	23	0	No toxicity
23	I	0.0	No acute	18	−0.8		Absent	24	0	No toxicity
24	I	0.0	No acute	25	−0.8		Absent	25	0	No toxicity
25	I	0.0	No acute	24	−0.2		Absent	9	22	Weak
2	II	33.3	Slight acute	2	1.9		Absent	2	22	Weak
6	II	33.3	Slight acute	9	3.2		Absent	13	22	Weak
11	II	33.3	Slight acute	17	3.4		Absent	15	22	Weak
13	II	33.3	Slight acute	20	4.8		Absent	17	22	Weak
17	II	33.3	Slight acute	11	4.9		Absent	18	22	Weak
18	II	33.3	Slight acute	13	5.8	3.4	Medium	19	22	Weak
20	II	33.3	Slight acute	21	6.1	3.4	Medium	20	22	Weak
21	II	33.3	Slight acute	22	6.1	3.4	Medium	1	33	Weak
22	II	33.3	Slight acute	6	6.9	3.4	Medium	6	33	Weak
7	II	66.7	Slight acute	1	7.9	3.4	Medium	11	33	Weak
10	II	66.7	Slight acute	15	8.2	3.4	Medium	12	33	Weak
16	II	50.0	Slight acute	19	8.7	3.4	Medium	22	33	Weak
1	III	33.3	Acute	16	9.5	3.4	Medium	16	39	Moderate
12	III	33.3	Acute	10	9.6	3.4	Medium	7	44	Moderate
15	III	33.3	Acute	12	10.2	3.4	Medium	5	56	Moderate
19	III	33.3	Acute	7	12.5	3.4	Medium	10	56	Moderate
5	III	55.6	Acute	14	12.8	3.4	Medium	14	56	Moderate
14	III	66.7	Acute	5	21.0	3.4	Medium	21	56	Moderate
3	IV	55.6	High acute	3	22.7	3.4	Medium	3	78	Strong
4	IV	88.9	High acute	8	34.1		High	8	89	Strong
8	IV	88.9	High acute	4	37.3		High	4	100	Strong

Abbreviations: SCF, statistical correction factor; TBI, toxicity test battery integrated index.

Dańko, & Kmita, 2016) found elevated concentrations of heavy metals (e.g. nickel and copper), DOC, total dissolved solids (TDS) and benzene, toluene, ethylbenzene, and xylenes compounds in sand with a larger fraction of reclaimed sand.

The high variability of the samples, including differences in the molding type and binder systems, could be enumerated among the limitations of this study. This variability acted as a confounding factor and hindered the establishment of a strong relationship between industrial processes and ecotoxicological results. Although the variability was recognized as a limitation, it was managed through the application of the classification systems.

Another interesting aspect is the comparison of the classification of undiluted and diluted leachates in the same system (Figure 4): No significant differences in the number of samples per class were observed, especially for the EC (Figure 4C, D). Indeed, in the undiluted and diluted conditions, the same three samples (23, 24, 25) were classified as "nontoxic," 12 out of 13 samples as "slightly toxic," five samples (5, 7, 10, 14, 16) as "moderate," and two samples (4 and 8) as "highly toxic." From an ecotoxicological point of view, even if the analysis of diluted samples could be more accurate, the substantial similarity in ranking between undiluted and diluted leachates may suggest the possibility of using only the undiluted leachate analysis, at least to

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TABLE 5 Classification of the diluted waste foundry sand leachates according to the Toxicity Classification System and the EcoScore system

Toxicity Classification System				EcoScore system		
Sample #	Toxicity class	Class weight score %	Toxicity	Sample #	EcoScore	Intensity of toxicity
9	I	–	No acute	23	0	No toxicity
23	I	–	No acute	24	0	No toxicity
24	I	–	No acute	25	0	No toxicity
25	I	–	No acute	9	6	Weak
20	I	33.3	No acute	18	8	Weak
2	II	33.3	Slight acute	13	11	Weak
6	II	33.3	Slight acute	15	14	Weak
12	II	33.3	Slight acute	6	17	Weak
13	II	33.3	Slight acute	22	17	Weak
15	II	33.3	Slight acute	19	20	Weak
17	II	33.3	Slight acute	2	22	Weak
18	II	33.3	Slight acute	12	22	Weak
22	II	33.3	Slight acute	21	22	Weak
16	II	33.3	Slight acute	17	22	Weak
21	II	66.7	Slight acute	20	22	Weak
1	III	33.3	Acute	1	33	Weak
5	III	33.3	Acute	16	39	Moderate
19	III	33.3	Acute	11	42	Moderate
11	III	50.0	Acute	14	44	Moderate
14	III	66.7	Acute	5	56	Moderate
3	III	83.3	Acute	7	64	Moderate
7	IV	55.6	High acute	10	67	Moderate
10	IV	66.7	High acute	3	67	Moderate
4	IV	77.8	High acute	8	92	Strong
8	IV	77.8	High acute	4	95	Strong

determine which samples are hazardous and which are not. This approach should facilitate a more cost-effective and time-efficient screening of raw materials, particularly those intended for unencapsulated applications (e.g., embankment and road subbase). These applications have the greatest potential for the release of a material and its constituents because the material is not chemically or physically bound, unlike the encapsulated reuse of WFS (e.g., in concrete, ceramics, glass). Encapsulation could prevent water from percolating through the foundry sand and minimize the potential for leaching and its impact on the environment (USEPA, 2014).

From a broader perspective, beyond the sole ecotoxicological assessment, the recovery of WFS is a matter of concern because of the impact of sands on both the environment and human health. It is evident that the entire life cycle of sands should be considered when analyzing their environmental impact. Several authors have identified three main impact categories: climate change, particulate matter, and resource use (Monteleone et al., 2024; Yiğit, 2013). According to Ghormley et al. (2020), a significant contributor to the impact of sand is its transportation, from the quarry to the foundry and then from the foundry to the landfill, due to the considerable distance to be covered. Mitterpach et al. (2017) suggested that WFS should be treated at the local level.

From the perspective of human health, the primary impacts are related to the working environment, because toxic and carcinogenic compounds are released during the sand preparation and the pouring process (Humfrey et al., 1996; Liljelind et al., 2010). Because residues of these chemicals may remain in the WFS fraction, it is advisable to include

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TABLE 6 Summary of the characteristics of nontoxic and highly toxic waste foundry sand samples

	Sands							Leachates	
				Type of molding		Binder system			
Ecotox classification	Sample #	Metals cast	Virgin sand used	Green	Resin	Core	Shell	pH	EC (µS/cm)
No toxicity	23	Iron	Silica		✓	Furan no-bake	Furan no-bake	5	483
	24	Iron	Silica		✓	Shell molding	Shell molding	7	12.5
	25	Iron	Silica	✓		Bentonite	Furan no-bake; Phenolic no-bake	7	190.5
High toxicity	4	Steel	Silica, zirconium, chromite, cerabeads		✓	Sodium silicate—ester cured	Phenolic/urethane no-bake; Ashland; Shell molding	8	1756
	8	Steel	Silica, chromite		✓	Sodium silicate—ester cured	Sodium silicate—ester cured; Phenolic/urethane no-bake	8	1290

Abbreviation: EC, EcoScore system.

mechanistic tests in the analytical battery to study the genotoxicity and mutagenicity of the WFS. This would be in line with the analytical schedules of waste, as proposed by other authors (Ferrari et al., 1999; Weltens et al., 2014). The lack of genotoxicity evaluation is a limitation of this study, and further studies should be conducted to address this gap.

Nevertheless, the principal objective of this study was to expand the existing WFS knowledge base. Ginsberg et al. (2019) stated that the newly acquired information should be employed "in a public health protective manner, even if the new information cannot at this point be converted into a quantitative prediction of population health risk."

A final consideration should be made regarding the whole approach described. Because the biotests are not designed to be substance specific, their application allows the assessment of the effects of an in toto mixture. This is the key aspect of such a biological approach because it facilitates the detection of biological effects related to interactions between living organisms and undetectable substances, synergistic or antagonistic effects of molecules, toxicokinetic and toxicodynamic phenomena, independently of the chemical characterization of a sample. The aim of this study was also to reverse the conventional principle that the results of chemical analyses are unequivocal, whereas biological data can be uncertain. Notably, biological assays are subject to a certain degree of intrinsic variability, which can be attributed to several factors. These include biological and experimental variables such as the genetic variability of the organisms and the location and operator ability, respectively. Nevertheless, this variability can be managed and turned to advantage by using several organisms, different endpoints, standardized procedures, and by combining the data obtained from the single test into synthetic indices that tell as much of the true story as possible. As stated by Prato et al. (2015) in the context of marine sediment quality assessment, if the results of the synthetic classification are interpreted as an integrative assessment, then there is a clear opportunity to prioritize samples of greatest concern.

CONCLUSION

In this study, the toxicity of WFS leachates was assessed using three ecotoxicity tests. The results were summarized using three different synthetic indices to assess the environmental risks associated with the possible reuse of these materials. According to the final classification, the samples of cast steel and a binder system based on sodium silicate-ester cured were very toxic and were associated with a high environmental risk, whereas the samples of cast iron and different binder systems were associated with a very low level of risk.

Classification systems are useful tools for responding to management and regulatory frameworks because they facilitate the visualization and synthesis of various hazards. Despite these findings, there is still a lack of development in

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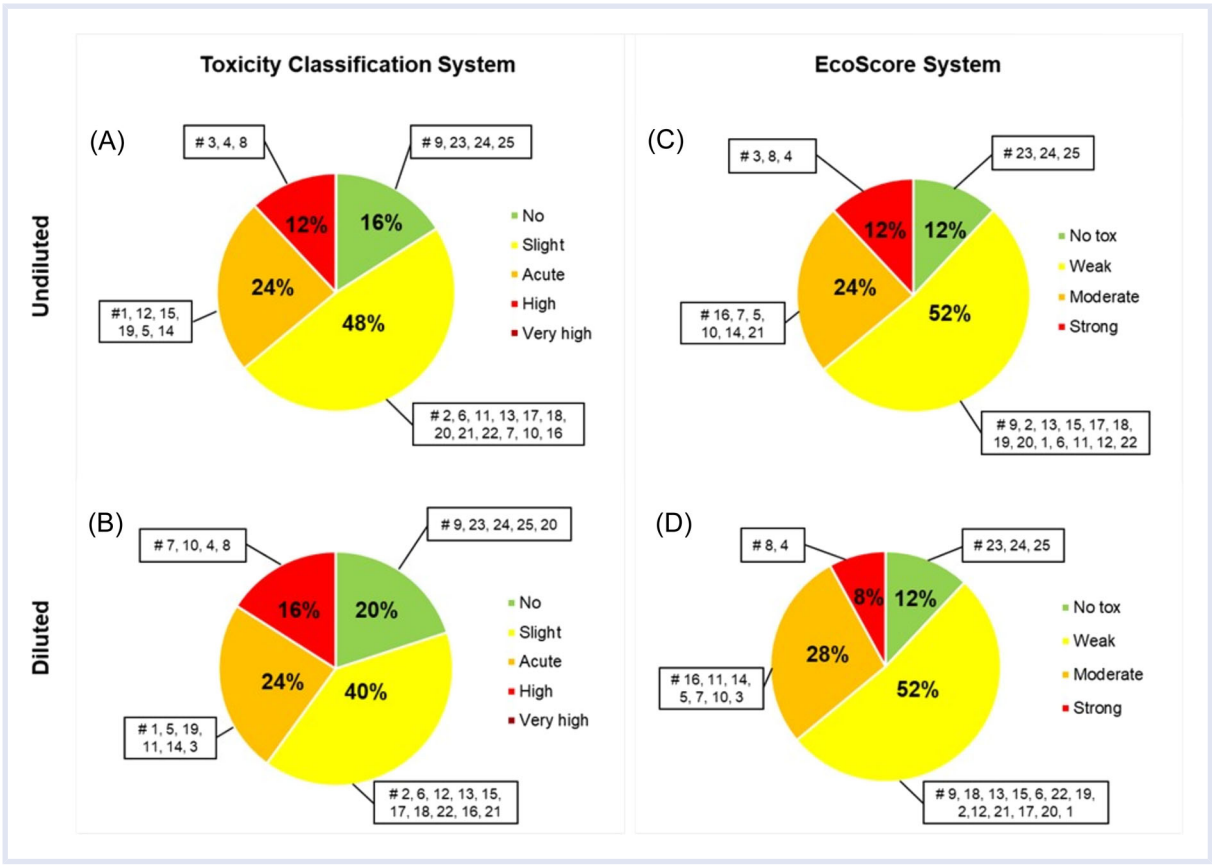


FIGURE 4 Comparison between the classification of undiluted (A and C) and diluted (B and D) waste foundry sand (WFS) leachates according to the Toxicity Classification System and the EcoScore system

the real-world use of these systems in the waste management industry.

Further studies are required to determine whether these classification systems can be used for evaluating matrixes other than those for which they were intended. The proposed approach may be included in strategies for reducing "the WFS" negative environmental effects and may be useful for long-term management of these materials, as well as a variety of other industrial wastes.

AUTHOR CONTRIBUTION

Carlotta Alias: Conceptualization; Formal analysis; investigation; methodology; visualization; writing—original draft; writing—review and editing. **Flavio Cioli**: conceptualization; investigation; writing—review and editing. **Alessandro Abbà**: Conceptualization; methodology, supervision, writing—review and editing. **Donatella Feretti**: Conceptualization; methodology, supervision; writing—review and editing. **Sabrina Sorlini**: Conceptualization; funding acquisition; methodology; project administration; supervision; writing—review and editing.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY STATEMENT

Data, associated metadata, and calculation tools are available from corresponding author Carlotta Alias (carlotta.alias@unibs.it)

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SUPPORTING INFORMATION

Detailed description of classification methods applied; raw physico-chemical data, photografical documentations of samples.

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