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BAUXITE MANUFACTURING RESIDUES FROM GARDANNE (FRANCE) AND PORTOVESME (ITALY) EXERT DIFFERENT PATTERNS OF POLLUTION AND TOXICITY TO SEA URCHIN EMBRYOS

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Abstract—This study was designed to investigate the composition and toxicity of solid residues from bauxite manufacturing plants. Soil and dust samples were collected in the proximity of two bauxite plants (Gardanne, France, and Portovesme, Italy). Samples were analyzed for their content of some selected inorganic contaminants by means of inductively coupled plasma optical emission spectroscopy (ICP-OES) either following acid digestion procedures or by seawater release of soluble components. Toxicity was tested by sea urchin bioassays to evaluate a set of toxicity endpoints including acute embryotoxicity, developmental defects, changes in sperm fertilization success, transmissible damage from sperm to the offspring, and cytogenetic abnormalities. Inorganic analysis showed two distinct sets of inorganic contaminants in Gardanne versus Portovesme, including Al, Cr, Cu, Fe, Mn, Pb, Ti, and Zn; sample composition (seawater-soluble contaminants) and toxicity showed a noteworthy association. The most severe toxicity to embryogenesis and to sperm fertilization success was exerted by some Portovesme samples (0.03–0.5% w/v), with a significant association between toxicity and dose-related seawater release of Zn, Pb, and Mn. Seawater extraction of a toxic dust sample (G20) from the Gardanne factory showed increasing seawater release of Al, Fe, and Mn; the G20 sample, at the level of 0.5%, affected both developing sea urchin embryos and sperm (offspring quality). Soil samples around the Gardanne factory showed the highest frequency of toxic soil sites eastward from the factory. The present data point to solid deposition from bauxite plants as a potential subject of environmental health concern. The results suggest that extraction methods for evaluating the toxicity of complex mixtures should be based on the environmental availability of mixture components. The differences in sample toxicity among the tested sites, however, suggest possible site-to-site variability in geochemical and/or technological parameters.

Keywords—Bauxite manufacturing Solid residues Sea urchins Soil toxicity Inorganic pollution

INTRODUCTION

Bauxite manufacturing involves ore processing in strongly alkaline medium (Bayer process) leading to the production of alumina (Al_2O_3). A process by-product is a red sludge whose disposal has been carried out either in marine coastal sites or in inland sites. As part of the sludge production, solid residues occur from sludge drying and from other process materials (crushed ore and reactor waste), spreading from bauxite plant facilities to the surrounding areas [1]. Solid residues from bauxite manufacturing either may occur as dust deposition in or closely around plant facilities or may be spread at longer distances and contaminate urban or land soil.

Previous investigations have focused on bauxite manufacturing sludge either as plant effluent [2,3] or as marine sediment from a coastal disposal site (Pagano et al., unpublished data). Aluminum bioaccumulation has been reported in marine plants collected nearby a coastal bauxite sludge disposal site [4], and adverse effects on a benthic macroinvertebrate community were also reported in a study of a bauxite sludge-polluted lake [5].

The issue of bauxite manufacturing by-products may be viewed as a subject of environmental concern, as bauxite by-products may affect either marine or freshwater benthic communities in the vicinity of sludge disposal sites [2–5]; (G. Pagano et al., unpublished data).

Thus, it appeared worthwhile to investigate whether solid residues released by some bauxite manufacturing plants could be associated with any comparable toxicity as was detected for bauxite sludge. A first sampling campaign was undertaken in May 1996 in Gardanne (southern France), and the early results showed relevant dust and soil toxicity on sea urchin early development. Subsequently, an expedition to Portovesme (Sardinia, Italy) in June 1998 provided further evidence that solid deposition from bauxite plants is, to a varying extent, associated with inorganic pollution as well as toxic outcomes. The present study provided confirmation of the working hypothesis of bauxite residues toxicity as well as some mechanistic insights about the possible roles of both the major bauxite components (Al and Fe) and minor components, such as Zn, Mn, and Pb.

The utilization of sea urchin bioassays in toxicity testing unconfined to marine pollution may be justified by the following facts. First, since the earliest reports, sea urchin embryos have been used either to evaluate marine pollution (e.g., seawater or sediment quality) or to test toxicity of a number of xenobiotics and nonmarine complex mixtures (e.g., pharmaceutical drugs or plant effluents), reviewed in [6,7]; in addition, in this series of studies, the red sludge may be viewed

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Fig. 1. Sampling sites of bauxite solid residues were located in (**a**) Gardanne (southern France, approximately 50 km north of Marseille) and (**b**) Portovesme (Italy, southwest Sardinia).

either as an aquatic or as a terrestrial pollutant, according to whether it is disposed offshore or in a lake or whether it is released in dry form as a solid residue from the plant [2–5]. Hence, both the extensive toxicological background studies and their continuity with our previous studies of bauxite sludge prompted us to verify whether and to what extent bauxite solid residues exerted any comparable toxicity to sea urchin embryogenesis.

MATERIALS AND METHODS

Sample localization and processing

The geographic localization of sampling sites is reported in Figure 1. Sampling operations were conducted outside the factories, with some access restraints caused by either the coastline (Portovesme, Italy) or abandoned inaccessible landfills (northwest of Gardanne, France). Six samples were collected in Portovesme along the track of a cable car connecting the plant with the waste disposal dock, from 5 to 150 m from the seaside. A circle-shaped sampling area was defined in Gardanne, including a total of 27 sampling sites, located as shown in Figure 2. Samples were collected around the local bauxite manufacturing plant, in a ray of 1 km that included the town of Gardanne, its suburbs, a power plant, and some agricultural areas. No access was available in a dumping site located northwest of the town (no sampling sites in Fig. 2). Dust and soil were collected as surface layers by means of either a brush or a shovel, respectively. The samples were stocked in 150-ml polystyrene containers and labeled with random numbers defining the coordinates of the site. At the laboratory, each specimen was filtered through a 1-mm sieve, then dried at 60°C for 48 h.

Chemical analysis

Overall metal content in each sample was analyzed by destructive determinations after complete dissolution by microwave disaggregation and chemical attack. Specifically, 0.1 g of each sample was contacted with 15 ml of a mixture of concentrated strong acids (HNO_3, 5 ml; HCl, 3 ml; and HF, 7 ml) in sealed polytetrafluorethylene (PTF) vessels (bombs). After insertion of the bomb into the microwave digestion system, MDS2100S from CEM (Matthew, NC, USA), the latter was operated at 60% of its maximum power for 15 min. The homogeneous digested solution was directly injected into the inductively coupled plasma optical emission spectroscopy (ICP-OES) system for metal analysis [8,9].

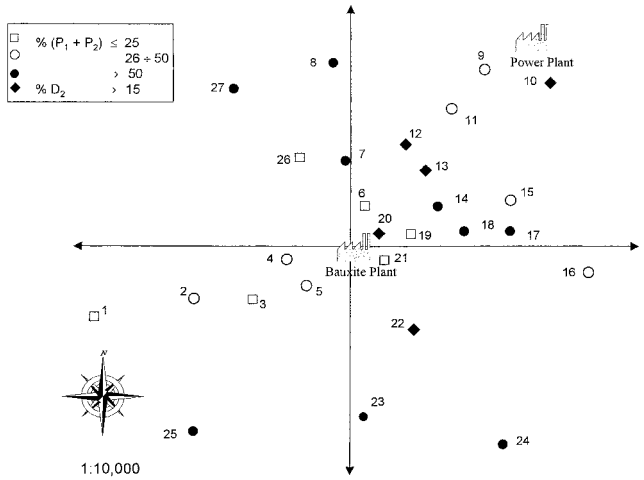

Fig. 2. The topographic distribution of soil and dust samples in Gardanne, France, was centered at the bauxite factory and included the town center and residential and agricultural areas. A coal-fueled power plant was located at the northeast border of Gardanne.

In order to evaluate seawater release of inorganics, the six samples from Portovesme and G20 sample from Gardanne (dust collected at the northern side of factory fence) were dried to constant weight and contacted with seawater (100 ml) in a Jar Test System F.6/S from Velp Scientific (Cambridge, UK) and stirred continuously (100 rpm) for 24 h. The supernatant solutions, after filtration on 0.45-μm polycarbonate, were analyzed for the content in seawater-released metals by ICP-OES spectrometry on a model Optima 3000 system from Perkin Elmer (Norwalk, CT, USA).

Sea urchins

Sea urchins from the species *Sphaerechinus granularis* (Desor) were utilized; gametes were obtained, and embryo cultures were run as described previously [6,7]. Controls throughout experiments were conducted as untreated negative controls (filtered seawater [FSW]) and 2.5×10^{-4}M $CdSO_4$ as a positive control [10]. Test samples were suspended at concentrations ranging from 0.01 to 0.5% (dry wt/v). Each test was run on six replicates of different embryo lots in six-well plates (Nunc, Roskilde, Denmark). The exposure of embryos (~100 embryos/ml) occurred throughout development from zygote (10 min after fertilization) up to the pluteus larval stage (72 h after fertilization). This procedure allows for a direct contact throughout cleavage up to hatching (~10 h after fertilization).

Sperm bioassays were conducted on sperm cell suspensions by a standard exposure of a 0.2% suspension of dry sperm pellet for 10 min. During exposure, test pellets were allowed to settle, and 10 μl of sperm-containing supernatant inseminated 10 ml of untreated egg suspensions (50–100 eggs/ml).

Table 1. Prevalence (%) of larval retardation (R) and developmental arrest (P2) in *Sphaerechinus granularis* embryos reared in bauxite plant particulate (G20) from Gardanne, France

No. treatment schedule	%	R	P2
Blank		7.0 ± 2.0	1.0 ± 1.0
G20	0.1	10.0 ± 4.0	7.0 ± 3.0
	0.5	37.5 ± 8.5	29.0 ± 3.0
	1.0	Cytolysis	

Thus, the offspring were cultured in nondevelopmentally toxic agent levels. Changes in the fertilization success of exposed sperm were determined by scoring percentage of fertilized eggs in fresh cleaving embryos (1–3 h postfertilization).

The observations of larvae with developmental defects were performed on living plutei immobilized in 10^{-4}M chromium sulfate for approximately 72 h after fertilization [6,11]. The following outcomes were evaluated: retarded (R) plutei (≤ half size vs normal [N] plutei); pathologic (P1) malformed plutei; pathologic embryos (P2), unable to differentiate up to the pluteus larval stage; and dead (D) embryos/larvae (scored as dead plutei [D1], or early dead embryos [D2]). All observations were carried out double blind by trained readers, each evaluating a complete set of readings.

Cytogenetic analysis

The sea urchin embryos at the stage of 64 to 128 cells were fixed in Carnoy's fluid (absolute ethanol:chloroform:acetic acid = 6:3:1) during 15 min. Then the fixative fluid was changed for absolute ethanol. An amount of approximately 200 μl of embryos pellet was dropped on a ice-cold slide, left to dryness for approximately 1 h, and stained by acetic carmine for 10 min. Dye excess was removed by rinsing in 30% acetic acid that was removed after 20 min, and then cover slides were placed. Double-blind observations were carried out by immersion (100×) objective. Mitotic abnormalities included changes in quantitative and morphologic parameters [6,7].

Statistical analysis

The outcomes were evaluated statistically using the Student's *t* test, applying the average square root transformation to normalize distributions. Those variables that were unsuitable for a parametric approach (from cytogenetic analysis) were evaluated with nonparametric tests: χ^2 test and Mann–Whitney *U* test. To carry out several simultaneous comparisons, Tukey's and Bonferroni's methods were used. Data analysis was carried out using the SPSS® for Windows statistical software [12,13].

Possible occurrence of correlations between observed developmental defects and metal concentrations measured in sediment samples was established by running correlation routine procedures (Pearson product–moment correlation) using Statistica® Software (StatSoft, Tulsa, OK, USA).

Table 2. Prevalence (%) of fertilization success (FR) and offspring quality (R and P2) following *Sphaerechinus granularis* 10-min sperm exposure to G20 particulate

No. treatment schedule	%	FR	R	P2
Blank		52.0 ± 5.0	11.8 ± 7.4	5.4 ± 1.9
G20	0.1	44.0 ± 7.0	13.6 ± 4.7	19.0 ± 15.3
	0.5	33.0 ± 7.0	23.0 ± 5.9	25.0 ± 3.1
	1.0	33.0 ± 5.0	11.8 ± 5.2	36.0 ± 11.4

Table 3. Concentrations of some selected inorganics from Gardanne, France, dust (G20) sample following strong-acid extraction (SAE [as mg/kg]) seawater extraction (SWE [as μg/L]) at the levels of 0.2 and 1 g/100 ml for 24 h. A bauxite sample was also analyzed by SWE. Measurements were carried out at the wavelengths specified below. Other elements were at levels below detection limits (μg/L): Cd < 2, Ni < 3, Pb < 30, Sb < 30, and Te < 30

Samples	Extraction procedure	Al (λ = 396.2)	Cu (λ = 324.8)	Fe (λ = 238.2)	Mn (λ = 257.6)	Zn (λ = 213.8)
G20	SAE	117,047	15	31,102	177	103
	SWE 0.2	45	6	14	6	3
	1.0	114	7	101	31	4
Bauxite	SWE 0.2	241	6	166	3	2
	1.0	244	7	190	3	3

RESULTS

Gardanne, France

A preliminary series of experiments was conducted on the G20 sample, consisting of light-brown dust collected at the factory fence. The first experiments were carried out on developing sea urchins at the 1% concentration and led to early embryolethality. The G20 sample was then tested on *S. granularis* embryos or sperm at lower concentrations, 0.1 to 1%. Developmental toxicity, as shown in Table 1, was evident at 0.5% G20 concentration, leading to 66.5% larval abnormalities, either as percentage retarded (R) larvae (37.5 ± 8.5) or as developmental arrest at blastula/gastrula stage (P2) (29.0 ± 3.0). When *S. granularis* sperm were exposed to the G20 sample, as shown in Table 2, a significant spermiotoxic effect was exerted at the concentration of 0.5% that also resulted in a dramatic increase of both delayed and arrested embryogenesis (R + P2 = 48%) in the offspring.

Based on the results of the G20 sample, a series of bioassays was conducted on the whole set of 27 samples collected in Gardanne, consisting of dust (G20) or soil surface from town gardens or agricultural soil. As shown in Figure 2, most of the toxic samples were enclosed within an approximate 120° angle east of the bauxite plant, including the power plant.

The inorganic analysis of G20 sample, as shown in Table 3, was carried out both by strong-acid digestion and by seawater extraction at the concentrations of 0.2 and 1 g/100 ml. The results showed that Al and Fe were the prevailing elements, as expected. In terms of seawater release, both Al and Fe and, to a lesser extent, Mn were dissolved at increased levels in seawater from the G20 sample, whereas the bauxite sample released substantial Al and Fe levels that, however, did not change by seawater extraction at 0.2 or 1%, respectively. Only minor (e.g., Zn) or undetectable levels of other inorganics (e.g., Pb) were found in both bauxite and G20 samples, following either strong-acid or seawater extraction (Table 3). The inorganic analysis of the whole set of 27 soil samples from Gardanne was run only by strong-acid digestion, as reported in Table 4. This data set, however, failed to provide any association between sample toxicity and nominal concentrations of individual analytes determined following strong-acid extraction.

Table 4. Inorganic analysis of Gardanne, France, soil samples (G1–G27) and a local bauxite sample conducted following strong-acid extraction. Concentrations are expressed as mg/kg ± standard deviation

Site no.	Al	Fe	Cd	Cr	Cu	Pb	Ti	Zn
G1	5,366 ± 656	9,081 ± 896	0.7 ± 0.1	10.1 ± 0.9	11.3 ± 1.0	33.9 ± 2.8	2.1 ± 0.3	39.9 ± 2.9
G2	11,595 ± 3,585	28,251 ± 1,666	1.0 ± 0.1	11.3 ± 4.5	14.0 ± 3.7	94.5 ± 6.7	2.8 ± 1.1	50.4 ± 18.0
G3	15,158 ± 853	35,139 ± 1,202	0.5 ± 0.0	9.6 ± 0.5	8.1 ± 0.4	29.7 ± 3.2	1.1 ± 0.1	29.6 ± 1.6
G4	11,071 ± 693	34,653 ± 2,764	0.6 ± 0.1	15.8 ± 1.2	6.9 ± 0.7	22.2 ± 2.9	1.2 ± 0.2	20.4 ± 1.7
G5	5,387 ± 426	8,772 ± 648	0.6 ± 0.0	7.0 ± 0.5	5.3 ± 0.4	23.9 ± 4.2	1.2 ± 0.0	22.1 ± 1.5
G6	14,332 ± 2,094	9,110 ± 1,822	1.2 ± 0.0	20.4 ± 4.2	18.8 ± 0.8	106.9 ± 17.9	20.6 ± 3.6	390.0 ± 30.4
G7	4,391 ± 1,149	9,779 ± 1,994	0.8 ± 0.1	11.1 ± 1.7	11.1 ± 2.4	60.7 ± 15.1	2.8 ± 0.7	55.5 ± 10.7
G8	5,654 ± 982	14,801 ± 1,806	0.7 ± 0.1	9.2 ± 1.2	9.7 ± 1.3	27.2 ± 3.9	1.4 ± 0.3	27.4 ± 3.8
G9	3,737 ± 1,516	6,134 ± 2,481	1.1 ± 0.1	10.7 ± 3.0	13.6 ± 4.8	39.3 ± 10.3	2.0 ± 0.1	31.6 ± 14.8
G10	9,188 ± 252	14,854 ± 383	0.7 ± 0.0	7.8 ± 0.2	9.6 ± 0.5	28.4 ± 1.2	1.3 ± 0.1	26.3 ± 1.2
G11	8,578 ± 447	16,085 ± 1,000	0.9 ± 0.1	11.5 ± 0.9	14.5 ± 0.8	35.7 ± 2.0	2.1 ± 0.4	48.9 ± 2.0
G12	1,186 ± 247	2,276 ± 1,344	1.2 ± 0.0	5.6 ± 0.5	12.6 ± 14.2	24.1 ± 1.6	2.7 ± 1.0	21.1 ± 8.1
G13	6,612 ± 171	16,427 ± 823	0.8 ± 0.1	9.9 ± 0.3	8.1 ± 0.4	26.8 ± 2.0	1.1 ± 0.1	30.2 ± 0.4
G14	13,252 ± 316	15,345 ± 587	1.0 ± 0.1	11.9 ± 0.5	12.7 ± 0.3	34.1 ± 0.5	2.0 ± 0.1	40.1 ± 0.7
G15	3,295 ± 702	5,652 ± 1,156	1.1 ± 0.1	8.9 ± 0.5	10.6 ± 1.4	92.1 ± 70.3	2.7 ± 0.4	36.6 ± 5.7
G16	4,989 ± 1,174	5,869 ± 1,043	1.1 ± 0.2	9.6 ± 1.9	9.7 ± 2.3	43.7 ± 9.4	6.2 ± 1.9	37.3 ± 11.0
G17	9,255 ± 4,448	9,433 ± 4,197	1.3 ± 0.1	12.2 ± 4.6	14.7 ± 4.7	53.9 ± 15.5	1.8 ± 0.1	40.4 ± 16.2
G18	5,831 ± 1,295	6,312 ± 1,624	1.2 ± 0.0	11.2 ± 2.5	18.5 ± 3.0	91.2 ± 15.4	5.3 ± 1.1	204.0 ± 41.4
G19	9,135 ± 400	14,473 ± 737	1.1 ± 0.1	15.7 ± 0.9	10.3 ± 0.2	53.0 ± 3.1	6.0 ± 0.7	47.1 ± 1.8
G20	4,843 ± 2,194	4,040 ± 1,736	1.0 ± 0.1	89.8 ± 39.3	14.1 ± 2.6	95.8 ± 39.3	65.6 ± 39.1	73.0 ± 29.3
G21	6,798 ± 1,578	9,675 ± 2,240	0.6 ± 0.1	7.6 ± 2.0	4.5 ± 1.1	19.7 ± 4.2	1.0 ± 0.6	14.1 ± 3.1
G22	3,993 ± 296	7,408 ± 751	0.9 ± 0.1	8.2 ± 0.8	5.9 ± 0.3	26.4 ± 0.4	1.9 ± 0.2	18.3 ± 1.3
G23	12,206 ± 1,550	28,447 ± 2,238	0.9 ± 0.0	10.6 ± 1.0	10.4 ± 1.2	37.0 ± 1.8	1.7 ± 0.2	33.8 ± 2.7
G24	5,548 ± 1,020	6,148 ± 1,250	0.9 ± 0.2	8.8 ± 1.8	11.0 ± 2.7	61.4 ± 12.2	1.2 ± 0.4	52.6 ± 10.2
G25	8,882 ± 1,530	14,365 ± 2,845	1.0 ± 0.2	10.3 ± 2.0	13.1 ± 2.6	41.5 ± 8.0	1.6 ± 0.2	34.1 ± 7.1
G26	9,491 ± 290	18,416 ± 797	2.0 ± 0.1	10.3 ± 0.7	10.7 ± 1.2	45.3 ± 5.0	2.7 ± 0.3	51.9 ± 7.9
G27	19,299 ± 1,916	24,364 ± 2,950	2.0 ± 0.0	15.4 ± 1.2	15.0 ± 1.1	64.7 ± 3.0	2.9 ± 0.9	35.4 ± 2.7
Bauxite	39,421 ± 8.171	Out of range	1.2 ± 0.0	155.7 ± 10.8	5.7 ± 0.3	48.9 ± 4.8	371.1 ± 25.5	9.2 ± 0.8

Portovesme, Italy

Soil sampling was carried out at Portovesme along the track of a cable car connecting the plant with the dock where bauxite residues are disposed and in the vicinity of the power plant serving the aluminum-producing factory. As shown in Table 5, developmental toxicity in *S. granularis* embryos was exerted by the two sampling sites closest to the seaside (5 and 15 m) and the site closest to the thermoelectric power plant (150PP), resulting in 100% developmental arrest (P2) at 0.5% sample concentration. Lesser effects, if any, were observed in embryos exposed to soil samples collected at 50 and 100 m from the seaside that failed to cause developmental arrest.

When *S. granularis* sperm were suspended in seawater with Portovesme soil samples at concentrations ranging from 0.01 to 0.1% (dry w/v), fertilization success was affected to a dramatic extent by the samples collected at 5 and 15 m from the seaside, as shown in Figure 3. The overall trend of spermiotoxicity for the different sampling sites showed minimal effects for the site at 100 m and, again, increased effects for the 150PP site. The effects on offspring quality, if any, could not be recorded for the spermiotoxic samples because of the extensive cytolysis of unfertilized eggs.

Inorganic analysis after strong-acid digestion of samples showed generally high Al and Fe levels that were, however, relatively low in the most toxic samples (e.g., site 5 and

Table 5. Prevalence (%) of developmental defects in *Sphaerechinus granularis* larvae reared in soil samples (0.5% w/v) collected below the cablecar track close to the Portovesme, Italy, bauxite plant at different distances from the seaside (industrial dock). The sample 150PP was collected at 150 m from seaside, close to the power plant (PP). Quadruplicate experiment. R = % retarded larvae; P1 = % malformed larvae; P2 = % embryos unable to undergo larval differentiation

Distance from seaside (m)	R	P1	P2
Blank	6.2 ± 2.9	3.7 ± 0.8	2.3 ± 0.4
Cd(II) 2.5×10^{-4} M	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0
5	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0
15	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0
50	10.3 ± 1.3	21.8 ± 1.3	9.8 ± 2.8
100	5.3 ± 3.1	10.0 ± 2.2	1.3 ± 0.6
150	1.5 ± 0.6	59.3 ± 19.5	39.3 ± 19.9
150PP	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0

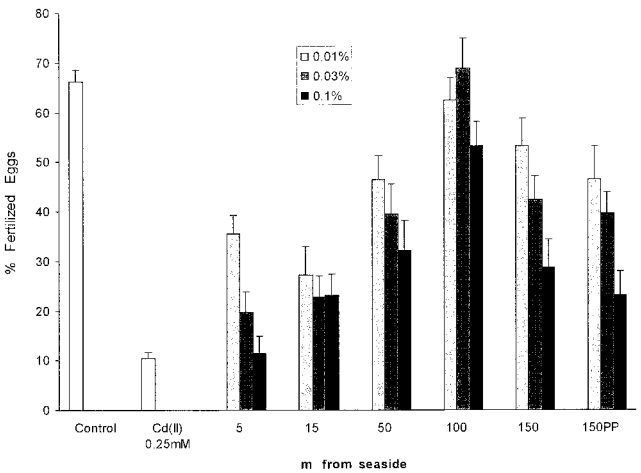

Fig. 3. Fertilization success of *Sphaerechinus granularis* sperm exposed to Portovesme, Italy, soil samples. The most severe spermiotoxicity was exerted by the sites closest to the coastline and by the 150PP site (nearest the power plant), consistent with the analogous embryotoxicity outcomes.

Table 6. Inorganic analysis of Portovesme, Italy, soil samples conducted following strong-acid extraction (concentrations are in mg/kg). Abbreviations as in Table 5

Distance from seaside (m)	Al	Fe	Cr	Cu	Mn	Ni	Pb	Zn
5	22,976	13,647	26	214	187	8	8,789	11,488
15	40,909	22,549	85	45	61	4	1,248	2,317
50	99,651	58,595	129	0	20	4	24	58
100	100,648	38,665	98	0	14	4	8	19
150	67,825	32,473	65	11	47	6	794	695
150PP	20,602	8,455	22	146	98	22	4,373	6,997

150PP), as shown in Table 6. On the other hand, minor inorganics showed their highest levels in the most toxic samples (Cu, Mn, Ni, Pb, and Zn). In particular, very high Pb and Zn levels were detected in toxic samples (5, 15, and 150PP), two orders of magnitude above their levels in relatively nontoxic samples, as in 50 and 100 (Table 6). The data obtained by strong-acid digestion were corroborated by seawater extraction of Portovesme samples at concentrations ranging from 0.1 to 2.0%. As shown in Table 7, both Al and Fe were detected in seawater at concentrations that did not change as a function of sample concentration. On the other hand, the levels of the other inorganics showed a steady increase related to sample concentrations that reached extremely high levels in the case of Zn, Pb, and Mn for toxic samples and were below detection limits in nontoxic samples. By processing toxicity outcomes in *S. granularis* embryos (R, P1, and P2) against each of these three metals (ln of concentrations), very good correlations were established for Zn ($r = 0.96$), Pb ($r = 0.77$), and Mn ($r = 0.84$).

Comparative toxicity testing

Some samples from both Gardanne and Portovesme were selected as having been relatively toxic or nontoxic when tested after their collection in May 1996 and June 1998, respectively. These samples were tested in the same experiment series in October 1999. The previously available data were confirmed in assessing the relative sample toxicities in the same locale, as, for example, 5 versus 100 m from seaside in Portovesme, or sample G20 versus S21 in Gardanne, as shown in Table 8. Moreover, the observed frequencies of developmental defects (P1 and P2) in larvae exposed to aged samples widely overlapped the corresponding data (Fig. 2 and Table 5) from the previous series of experiments dating back to approximately three and a half years and a year and a half earlier, respectively. Cytogenetic analysis was carried out on some selected samples from Gardanne and Portovesme on *S. granularis* embryos. Neither morphologic effects nor mitotoxicity were observed.

Table 7. Concentrations (as μg/L) of some selected inorganics from Portovesme, Italy, soil samples following seawater extraction at different pellet levels (0.1–2 g/100 ml). The following elements were invariably below detection limits (μg/L): As < 40, Cr < 4, Ni < 3, and Sb < 30

Distance from seaside (m)	% (seawater)	Al	Cu	Fe	Mn	Pb	Zn
5	0.1	82	<3	18	6	467	761
	1.0	100	<3	20	41	500	4,620
	2.0	98	17	25	68	537	5,360
15	0.1	221	<3	10	5	<30	102
	1.0	241	<3	12	15	65	280
	2.0	250	<3	13	25	114	486
50	0.1	100	<3	22	4	<30	<1
	1.0	100	<3	26	40	<30	<1
	2.0	110	<3	30	40	50	84
100	0.1	110	<3	11	3	<30	<1
	1.0	132	<3	20	40	<30	<1
	2.0	158	<3	28	46	<30	32
150	0.1	88	<3	10	3	<30	53
	1.0	100	<3	12	11	46	180
	2.0	104	4	12	35	69	316
150PP	0.1	86	<3	15	6	289	257
	1.0	92	<3	16	87	468	2,300
	2.0	120	12	18	111	663	3,530

DISCUSSION

Dust and soil samples from two bauxite manufacturing plants have been investigated in an effort to relate chemical composition of bauxite solid residues to their toxicity to sea urchin embryos and sperm.

The rationale for utilizing the sea urchin bioassay model in testing complex mixture toxicity not confined to marine sampling sites, as in the case of bauxite solid residues, first relies on the wide scope of applicability of sea urchin bioassays. These provided multiparametric information on an extensive set of various agents and complex mixtures (including marine, brackish, and freshwater sediment and industrial sludge and effluents) [2,6,7,14–16]. Moreover, sea urchin bioassays provide the possibility to test environmentally occurring mixtures with or without extraction procedures, which may lead to potentially unrealistic information [7]. Thus, sea urchin bioassays can be envisaged as a multipurpose tool in toxicol-

Table 8. Prevalence (%) of developmental defects in *Sphaerechinus granularis* larvae reared in some selected soil samples (0.5% w/v) from Portovesme, Italy, or from Gardanne, France. Quintuplicate experiment carried out in October 1999, one and a half and three and a half years after sample collection in Portovesme and Gardanne, respectively

Sample no.	R	P1	P2
Control	5.3 ± 1.0	5.7 ± 1.1	1.7 ± 0.5
$Cd(SO_4)_2$ 2.5×10^{-4} M	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0
Portovesme			
S011 (5 m)[a]	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0
S522 (15 m)	0.0 ± 0.0	3.0 ± 3.0	97.0 ± 3.0
S489 (100 m)	8.0 ± 2.9	14.8 ± 5.3	0.6 ± 0.4
Gardanne			
G10	10.8 ± 3.1	50.0 ± 10.8	5.6 ± 0.7
G12	16.0 ± 3.1	34.2 ± 8.3	8.8 ± 5.1
G20	2.6 ± 1.6	49.0 ± 12.9	39.6 ± 12.1
G21	11.0 ± 2.4	11.3 ± 3.7	4.8 ± 3.3

[a] Distance from seaside (m) as reported in Tables 5 to 7.

ogy allowing for the assessment of a multiple set of endpoints independently of the source of test materials, such as drugs, or industrial pollutants or complex mixtures [14–19].

The results of the present study provided support for the working hypothesis that bauxite solid residues, collected in the vicinity of bauxite manufacturing plants, might exert developmental and reproductive toxicity [19], consistent with our previous reports showing toxic effects of bauxite sludge to sea urchin and mussel sperm and embryos [2,3]. Those data were interpreted as due predominantly to aluminum and iron toxicity based on the analytical approach (strong-acid extraction) utilized at that time and on the massive Al and Fe levels in sludge compared to very low levels of the other elements analyzed for. Those previous analytical data are confirmed in the present report by comparing the sparing concentrations of minor metal contaminants in the Gardanne G20 dust sample collected at the factory fence (Table 4) as compared to the substantially different composition of Portovesme samples (Tables 6 and 7). The present study has provided evidence that the toxic effects from soil and dust samples from bauxite manufacturing plants may or may not be related to Al/Fe toxicity according to their environmental availability. In the case of Portovesme samples, the observed effects are derived mainly from the minor metal contaminants, especially Zn, Pb, and Mn, with a tight correlation between their levels and sample toxicity. By relating metal analysis to toxicity for soil and dust samples from two bauxite manufacturing plants, we have been able to elucidate more clearly the source(s) of toxicity in the bauxite residues.

In the case of Gardanne, most toxic samples were found in a sector of 120° east of the bauxite plant. This observation may be suggested to be consistent with the prevailing west winds in the region. The analytical data set presently available includes early data (1996) on the 27 sampling sites obtained by strong-acid digestion and recent data (1999) obtained from the G20 sample (out of factory) by both strong-acid digestion and seawater extraction. The present limitation in the analytical data set does not allow us to relate seawater-extractable inorganics levels and the toxicity data of the whole set of sampling sites. Even so, the available data from the G20 sample point to the presence of seawater-extractable Al, Fe, and Mn, whereas Zn and Pb fail to show any detectable increase in their seawater release. Hence, the environmental availability of these inorganics appears to be drastically different in the G20 sample compared to Portovesme samples.

This study confirms our previous findings [2,3] about the developmental toxicity to sea urchins of red sludge from bauxite manufacturing. This holds true for the Gardanne sludge and solid deposition, with environmental concern unrestricted to marine organisms in the vicinity of the coastal disposal site, but also potentially involving terrestrial biota. The characteristic, highly toxic dust from the G20 site collected at the factory fence outside the factory might represent a subject of environmental concern in the town of Gardanne, deserving appropriate investigations.

In the case of the Portovesme factory, a significant correlation was obtained when the observed toxicity was measured against the levels of minor metals in the bauxite residues, namely, Zn, Pb, and Mn. It is worth noting that the most toxic samples from Portovesme were effective in inducing developmental arrest (P2); moreover, these effects were detected at very low concentrations, ranging from 0.03 to 0.3%. It is also noted that samples 5, 150, and 150PP are very similar based on their inorganics concentrations, and samples 5 and 150PP had the same black color (unlike the others, which were reddish). It is suggested that, depending on the emission from the factory and wind direction, the 5 and 150PP samples shared a similar contamination, possibly including ashes and pyrolysis products from the power plant. This hypothesis should be verified in further studies. The possible role for other neighboring plants in causing the observed composition and toxicity of solid waste should also be investigated (G. Oggiano, personal communication).

Cytogenetic analysis failed to provide evidence for morphologic aberrations, yet it showed a significant decrease in metaphase:anaphase ratio, suggesting an alteration of the cytoskeleton. These data were in partial agreement with the cytogenetic effects of aluminum salts reported previously [15]. However, it should be noted that the effects of these complex mixtures may not reflect the effects of individual components.

The observed differences between seawater release of metals from G20 compared to Portovesme samples are not surprising in view of the established differences in the physicochemical properties of bauxite ores [1] that comprise a multitude of mineralogic forms and are characterized by different structures and chemical composition. As additional source of variability, differences in ore processing and waste handling might result in different composition and toxicity of solid residues [19]. Hence, the present information on the different behaviors of Gardanne versus Portovesme samples might represent only a first step of more complete information when data from other bauxite manufacturing plants becomes available.

CONCLUSIONS

This study first reports on analytical versus toxicity data of bauxite solid residues. Inorganic contamination and environmental availability of some metals were evaluated under different conditions, providing the following evidence: a set of multiple contaminants including Al, Fe, Mn, Pb, and Zn; a wide variation of sample composition within each sampling area, with a significant association between seawater-soluble contaminants and toxicity. The most severe toxicity of some Portovesme samples was associated with a dramatic dose-related seawater release of Zn and, to a lesser extent, Mn and Pb.

Seawater extraction of a dust sample (G20) from Gardanne factory showed increasing seawater release of Al, Fe, and Mn and affected developing sea urchin embryos and sperm (reflected by damage to offspring quality); the highest frequency of toxic soil samples within a circular set of sites around Gardanne was included in a 120° angle eastward from the factory, in agreement with the prevailing west winds in the region.

The results suggest that solid deposition from bauxite plants may be a subject that is broadly unknown and yet of environmental health concern. The present study suggests that the traditional strong-acid extraction methods for analyzing inorganic complex mixtures may provide misleading information, that is, not reflecting the actual release of soluble components from the complex mixtures. Realistic extraction methods not confined to strong-acid extraction would be useful in evaluating environmental availability and effects of complex mixtures. Differences in sample toxicity among and within selected sites suggest a variability in geochemical and/or technological aspects of treatment of the bauxite ores.

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