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Geospatial pattern of topsoil pollution and multi-endpoint toxicity in the petrochemical area of Augusta-Priolo (eastern Sicily, Italy)

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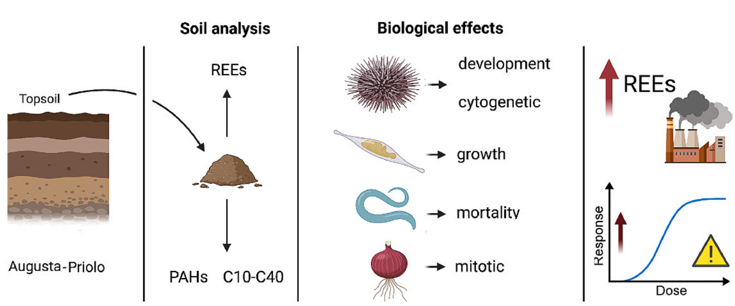
HIGHLIGHTS

- Topsoil contamination and toxicity was evaluated in a petrochemical area in Sicily.
- Inorganic and organic pollutant levels were measured at different distances from refineries.
- Multiple bioassays were carried out in testing topsoil toxicity.
- Consistent results were found in topsoil from urban areas vs. sampling sites.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study was aimed at identifying geospatial patterns of pollutants including concentrations and toxicity as complex environmental mixtures, in topsoil samples close to petrochemical facilities in the heavily industrialized area of Augusta and Priolo in south-eastern Sicily (Italy). Elemental analysis of soil was conducted by ICP-MS for 23 metals and 16 rare earth elements (REEs). Organic analyses were primarily focused on polycyclic aromatic hydrocarbons (PAHs) (16 parent homologs) and total aliphatic hydrocarbons (C10 – C40). Topsoil samples were tested for toxicity in multiple bioassay models including: 1) developmental defects and cytogenetic anomalies in sea urchin *Sphaerechinus granularis* early life stages; 2) growth inhibition of diatom *Phaeodactylum tricornutum*; 3) mortality in nematode *Caenorhabditis elegans*; and 4) induction of mitotic abnormalities in onion *Allium cepa*. Samples collected at sites closest to defined petrochemical facilities were highest in select pollutants and correlated with biological effects in different toxicity endpoints. A noteworthy finding was

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the increased level of total REEs in sites closest to petrochemical facilities, suggesting their contributions to identifying petrochemical sources of pollutants to the environment. The combined data obtained in the different bioassays allowed exploration of geospatial patterns of effect in biota as a function of contaminant levels. In conclusion, this study provides consistent data of soil toxicity, metal and REE contamination at Augusta-Priolo sampling sites, and may provide an appropriate baseline for epidemiological studies on high incidences of congenital birth defects in the area and identification of at-risk localities.

1. Introduction

Over recent decades, the petrochemical industry has come under increased scrutiny for its potential contribution to pollutant releases into the environment which may consequently have adverse effects on human and environmental health. While the concept of transitioning to a greener economy has been gaining ground over recent years, the phasing-out of fossil fuels presents multiple difficulties due to our reliance on energy provided from such sources and the lack of reliable alternatives. As petrochemical plants and refineries are oftentimes located in the vicinity of large population centers, and with the high potential for pollutant emissions, epidemiology and the monitoring of residents' health has gained great interest in many parts of the world.

In Asia, a study on residents who lived within 10 km from the largest petrochemical complex in Taiwan for at least 12 years showed a significantly greater incidence of cancers among females and older (>60 years of age) residents than those living 10–40 km from the plant (Yuan et al., 2018). Furthermore, a recent meta-analysis calculated an overall 36% increased risk of leukemia for those living within 8 km from petrochemical complexes, while specific subtypes such as acute myeloid leukemia and chronic lymphocytic leukemia showed even higher relative risk of 61% and 85%, respectively (Lin et al., 2019).

Indeed, apart from concerns regarding population health coming to the fore, reports on occupational exposures in petrochemical facilities have also found increased risk of negative impacts on health. In China it has been reported that women exposed to petrochemicals in a Beijing industrial complex had a 2.7–2.9 times higher incidence of miscarriage compared to non-exposed women in the same plant (Xu et al., 1998), while exposure in the gas and petrochemical industries was also shown to increase concentrations of metals such as arsenic, nickel, cadmium, manganese and vanadium in urine (Kafaei et al., 2019).

Among petrochemical industry workers in Italy it has been reported that malignant tumors of the pleura and peritoneum are approximately 6 times higher than in other industries, with incidence of respiratory system diseases about 2.5 times higher (Campo, 2013). In the Sicily region, as a component of Italian national epidemiological and public health surveillance programs, a range of adverse health outcomes has been monitored in various periods in the past, including reproductive health (Cernigliaro et al., 2016) and cancer incidence, hospitalization and mortality (Cernigliaro et al., 2019). In particular, in the town of Gela which is impacted by point sources of contamination such as chemical industry, petrochemical plant and/or refinery and landfill, analysis of congenital anomalies showed a 236% increased incidence of genital defects in newborns over that which would normally be expected in the 2006–2010 period (Santoro et al., 2017). These data are among some the highest levels of defects reported in the literature, and, with increased rates of respiratory disease and mortality from lung cancer, have led to the hypothesis that emissions from petrochemical and refinery complexes may be a key factor in driving these negative health outcomes (Pirastu et al., 2011).

To the east of Gela, at a distance of 80 km, lies the conurbation of Augusta-Priolo-Melilli (Fig. 1) whose combined facilities of three oil refineries and related petrochemical industries as well as a cement plant represent one of the largest industrial footprints of this sector in the whole of modern-day Europe, considering the extremely small geographical area in which they are compressed. In particular, it has been recently estimated that these industries combined currently produce more than 4.5 M tonnes CO_2 annually which is more than 1% of the total output of Italy (ClimateTrace, 2023), along with prevailing wind regime (Fig. S1). In fact, in the 2006–2010 period, it was noted that in newborns there was a 154% increased incidence of genital defects and 165% increased incidence of digestive system anomalies with respect to national baseline values (Santoro et al., 2017).

In parallel with human health considerations, a range of studies have been carried out near petrochemical facilities on the analysis of environmental samples (see, e.g., Di Leonardo et al., 2014) using a range of

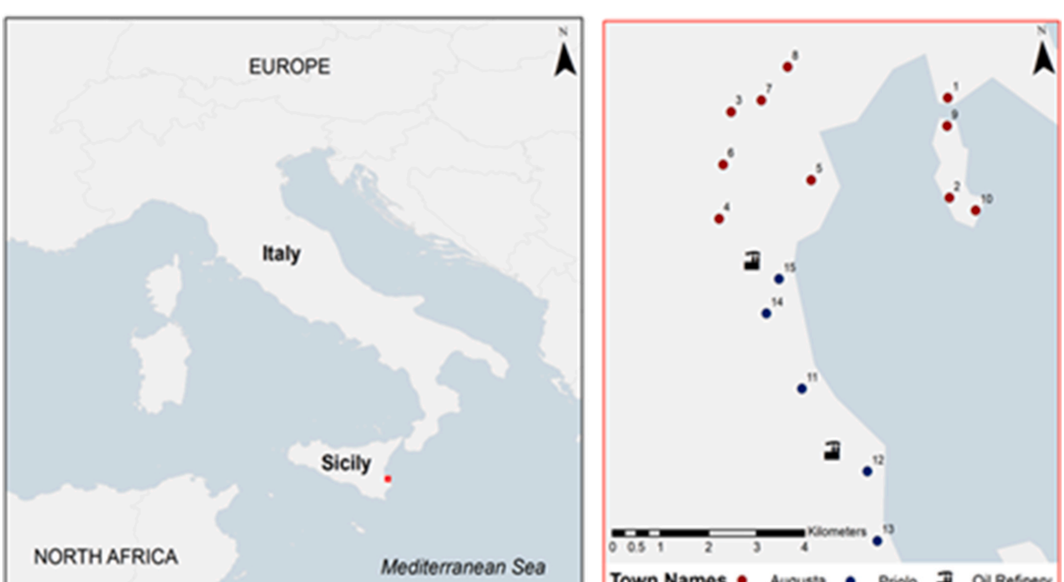

Fig. 1. An overview map of sample locations.

soil- and sediment-based bioassays (Li et al., 2009; Höss et al., 2009; González et al., 2013; De Domenico et al., 2013; Signa et al., 2017; Caricato et al., 2019; Copat et al., 2020) which have clearly demonstrated the deleterious impact of these environmental samples, particularly those with high metal and polycyclic organic concentrations, on a number of biota, including marine organisms and human health (Mudu et al., 2014; Maisano et al., 2017; Gorini et al., 2020; Romano et al., 2021) as summarized in Table 1. However, a relevant task in evaluating the possibly adverse effects of soil in proximity of petrochemical facilities needed an ad hoc investigation. This approach was the main objective of our present investigation, by continuing previous studies of soil contamination in other industrial areas, such as bauxite processing facilities (Pagano et al., 2002; Oral et al., 2019) and steel foundry (Trifuoggi et al., 2019).

As is the case for many other complex mixtures, knowledge on the effects and underlying mechanisms of toxicity in biota triggered by environmentally-relevant exposures has been mostly drawn from experimental research under laboratory conditions for single compounds where ecological relevance was often insufficiently considered in terms of concentrations, model organisms and, most importantly, chemical mixtures. In spite of the many compounds known to science, research had tended to focus on a few representative substances (e.g. USEPA priority PAHs), and not necessarily on most common compounds in a particular environmental setting, such as the one presented in the Augusta-Priolo context. Moreover, as toxicity data in a bioassay can be equivocal depending on the appropriateness of the model organism chosen, it is increasingly clear that a panel of bioassays are required to reliably determine an overall toxicity profile of environmental samples. On the basis of our hypothesis that not only proximity to a heavily industrialized area plays a key role in environmental exposure to pollutants but that microlocations which may be influenced by abiotic factors such as wind regime may be differentiated, we report herein a geospatial analysis of contaminant signatures and biological effects over a wide area around the Augusta-Priolo petrochemical facilities. Biological effects from exposure to whole sediment samples were modeled alongside the chemical fingerprints obtained through inorganic and organic analyses to evaluate whether or not there was an increased risk of environmental toxicity in the locality of the petrochemical facilities.

Table 1
Summarized evidence reporting on links of petrochemical industry products *vs.* analytical findings and adverse health effects.

Environmental analyses	Reported findings	References
Marine surface sediments	Occasional elevated concentrations of Hg, Cd, Ni and PAHs	Di Leonardo et al. (2014); Signa et al. (2017)
Levels and bioreactivity of $PM_{2.5}$	Excess emissions and bioreactivity a petrochemical complex	Chuang et al. (2018)
Metal levels	In soil close to petrochemical plant	Li et al. (2009); Relić et al. (2019)
Bioassays		
Stone curlew *(Burhinus oedicnemus)*	Trace element bioaccumulation	Copat et al. (2020)
Earthworm *(Eisenia andrei)*	Toxicity and bioavailability of a metal-contaminated soil	González et al. (2013)
Invertebrate and plant bioassays	Toxicity of petroleum-contaminated soil	Hentati et al. (2013)
Nematode *Caenorhabditis elegans*	Toxicity of petroleum-contaminated soil	Höss et al. (2009)
Springtail *Folsomia candida*	Toxicity of oil refinery waste	Reinecke et al. (2016)
Mytilus galloprovincialis	Histological changes, neurotoxicity and hypoxic stress	Maisano et al. (2017); Caricato et al. (2019)
Sea bass (*Dicentrarchus labrax*)	Altered functioning of the nervous and endocrine systems	De Domenico et al. (2013)

2. Materials and methods

2.1. Study area and sampling sites

The heavily industrialized study area is located between Augusta and Priolo (south-eastern Italy), with site coordinates given in Table 2, and includes two main oil refineries and a series of subsidiary facilities and harbors, with the area hosting a population of approximately 50,000 inhabitants (Fig. 1).

2.2. Topsoil collection and processing

Topsoil samples were collected with a steel shovel from road edges or from cultivated soil. Approximately 100 g of soil were collected at each location following previously published protocols (Woszczyk et al., 2018). Samples were sieved in 2-mm mesh steel nets, ground in a ceramic pestle and dried at 60 °C for 24 h, for running subsequent analyses and bioassays.

2.3. Metal, PAH and total organic analyses

Soil samples were analyzed for a set of elements, including 23 metals and 16 rare earth elements (REEs). For the determination of metals and REEs, soil samples were subjected to oxidative acid digestion using a mixture of nitric and hydrofluoric acid (0.25 g sample + 3.5 ml HNO_3 + 0.1 ml HF) at 180 °C and high pressure (800 psi), assisted by microwave (Mars, CEM, Italy) as reported previously (Oral et al., 2019; Trifuoggi et al., 2019). For solubilizing heavy metals, the US EPA 3050 B (1996) method was followed. The quantitative determination of trace elements was carried out using the UNI EN ISO 16171 (2016) method by

Table 2
Localization of sampling sites in Augusta and Priolo.

# Site	Location	Coordinates
Augusta		
1	Old City Gate	37° 14′ 153″ N, 15° 13′ 196″ E
2	Uncultivated land near the Port	37° 13′ 405″ N, 15° 13′ 112″ E
3	Industrial area (Punta Cugno)	37° 14′ 239″ N, 15° 10′ 113″ E
4	Outside the Refinery	37° 13′ 150″ N, 15° 09′ 562″ E
5	Sheepfold – Punta Cugno	37° 13′ 774″ N, 15° 10′ 937″ E
6	Waste-to-energy plant	37° 13′ 858″ N, 15° 81′ 307″ E
7	Outside Punta Cugno	37° 14′ 299″ N, 15° 10′ 379″ E
8	Outside commercial port (Plowed Agricultural Land)	37° 14′ 632″ N, 15° 10′ 847″ E
9	Public garden outside Swabian Castle	37° 13′ 943″ N, 15° 13′ 208″ E
10	East Coast (Dog Beach)	37° 13′ 315″ N, 15° 13′ 440″ E
Priolo		
11	Intermediate between two refineries	37° 11′ 227″ N, 15° 10′ 571″ E
12	W ENEL Refinery – Priolo Gargallo	37° 09′ 865″ N, 15° 11′ 485″ E
13	Suburbs Priolo	37° 09′ 418″ N, 15° 11′ 535″ E
14	Megara Hyblea (Archeological Site)	37° 12′ 125″ N, 15° 10′ 316″ E
15	Megara Hyblea 2	37° 12′ 324″ N, 15° 10′ 709″ E

inductively coupled plasma mass spectrometry (ICP-MS) carried out on an Aurora M90 instrument (Bruker, USA). The detection limit (LOD) and limit of quantification (LOQ) were calculated using the method of blank variability for each investigated metal. The calculated average values of LOD and LOQ were 0.06 and 0.16 mg/kg, respectively. QA/QC for metals and REEs were evaluated by using reference materials CR-CRM 667 and 277 R (European Commission Joint Research Centre, Geel, Belgium). The percentages of recovery were in the range 60–120%.

Soil samples were also analyzed for total hydrocarbons (C10 – C40) and PAH contents, as reported previously by Trifuoggi et al. (2019). The dry soil samples were extracted with a mixture of acetone/n-hexane (1:1 v/v) and sonicated for 3 h by ultrasonic disruptor. A part of the extract was purified with anhydrous sodium sulfate (preheated at 550 °C for 2 h) and analyzed by gas chromatography coupled with mass spectrometer (Shimadzu 2010 Plus and MS-TQ8030, Shimadzu, Japan) for PAH analysis. The detection limit (LOD) and limit of quantification (LOQ) were calculated using the range method of prediction using linear regression to the 95% level of probability for each investigated PAH. The calculated average values of LOD and LOQ were 0.002 and 0.005 mg/kg, respectively. Another part of the extract was purified on a florisil column (2 g florisil with granulometry of 60–100 mesh, activated at 150 °C for 24 h) and analyzed by gas chromatography with a Flame Ionisation detector (FID; Agilent 6890, USA) for the determination of total hydrocarbons (C10 – C40). The values of LOD and LOQ were 3 and 10 mg/kg, respectively. Data accuracy was checked by a certified reference material BCR-CRM 535 (freshwater harbour sediment, European Commission Joint Research Centre) for PAHs and for total hydrocarbon analysis, and method performance was also evaluated through participation in inter-laboratory circuits (UNICHIM, Italy). The percentages of recovery were 60–80% and 70–100% for PAHs and total hydrocarbons respectively.

2.4. Sea urchin assays

Specimens of *Sphaerechinus granularis* sea urchins were collected along the Rovinj coast by the staff of the Centre for Marine Research - Ruđer Bošković Institute (Rovinj, Croatia, 45°04′47″ N, 13°38′24″ E). Gametes were obtained, and embryo cultures were run as described previously (Pagano et al., 2017). Controls were run as untreated negative controls in natural filtered seawater (FSW; salinity S‧35, pH 8). No "pristine" soil control was utilized due to the expected – and verified – differences in soil toxicity that allowed for distinctions within the sample sets according to their topographic distribution.

For embryotoxicity tests, portions of topsoil were placed at the bottom of each well of 6-well tissue culture plates (Falcon™ 10 ml/well) and suspended in 9 ml FSW such that the soil concentration was 0.1% w/v. Thereafter, 1 ml of zygotes (10 min post-fertilization, p-f) was added to topsoil suspensions and incubated at 18 °C in the dark for 72 h. The embryos were thus exposed throughout development up to the pluteus larval stage, and subsequently scored for developmental defects.

Cytogenetic analysis was carried out on topsoil-exposed embryos (0.1% w/v, fixed 5 h p-f). Three endpoints were evaluated in mitotic activity of cleaving embryos, including: a) mean number of mitoses per embryo (MPE); b) % interphase embryos (IE), lacking active mitoses, and c) % embryos with $\geq$1 mitotic aberration (%Emb_Ab+). Thus, both mitotic inhibition (a, b) and aberration frequency (c) could be evaluated.

Sperm bioassays were performed on *S. granularis* sperm suspensions. A 100-μl aliquot of sperm was suspended in 10 ml of stirred topsoil suspensions (0.5% w/v). After 10 min, 100 μl of sperm from each suspension was used to fertilize untreated egg suspensions. The fertilization rate (FR) of exposed sperm was determined by scoring the percentage of fertilized eggs in live cleaving embryos (1–3 h p-f). Thereafter, at 72 h p-f, the offspring of exposed sperm were scored for developmental defects.

Each bioassay was run in six replicates. The scoring of larvae was performed on the first 100 plutei observed in each replicate which had been immobilized by the addition of 10^{-4} M chromium sulfate 5 min prior to observation (Pagano et al., 1983). Embryogenesis abnormalities were scored as: (i) pathologic (P1), malformed plutei; (ii) pathologic embryos (P2), arrested at pre-larval stages, and (iii) dead (D) embryos. The total percentages of P1 + P2 + D were scored blind by trained readers, each one evaluating a complete set of cultures.

2.5. Microalgae assays

Axenic cultures of *Phaeodactylum tricornutum* microalgae were cultured at the Hygiene Laboratory, Department of Biology at the University of Naples Federico II in artificial seawater medium. Algal growth inhibition test (72 h) was performed according to ISO 10253 (2016) using multiwell plates. Inocula of exponentially growing microalgae were exposed to elutriates (prepared 1:4 w/v according to the standard procedure of US EPA, 1991) spiked with nutrient stock solutions for 72 h at 22 ± 1 °C under continuous light of 6700 lux and with continuous shaking at 50 rpm.

Three replicates for control and each sample were prepared. After a 72-h exposure, spectrophotometric measurement of samples at 670 nm (DR 5000 sc, Hach) was performed to determine algal cell density. The growth rate relative to the control was calculated by normalizing the final cell density of each replicate by that of the control culture, assumed as 100%.

2.6. Nematode assays

Assays were carried out on cultures of the nematode *Caenorhabditis elegans* wild-type strain N2 variant Bristol, cultured at the Hygiene Laboratory, Department of Biology at the University of Naples Federico II, in nematode growth media (NGM) plates seeded with *Escherichia coli* (strain OP50-Uracil deficient. The nematode acute test was carried out according to the ASTM E2172 – 01 Standard Method (2014). The tests were performed using age-synchronous adult nematodes and achieved via bleaching (Egg Prep) according to Race et al. (2019). Soil samples (2.33 g) were hydrated to 35–45% of their dry weight with K-medium in centrifuge tubes. Ten worms were transferred to each test tube and exposed for 24 h at 20 °C to the soil samples. Four replicates for control and each sample were prepared. Nematodes were then recovered from the soil samples by gentle centrifugation through a colloidal silica suspension (Ludox TM 50, Sigma-Aldrich, St. Louis, MO, USA) which allows floating the nematodes on top of the suspension. The recovered individuals were then counted under a microscope (40 × magnification) with mortality as the measured endpoint.

2.7. Bulb onion assays

Bulb onions *Allium cepa* L. from commercial sources were stored in a cold room for at least seven days and were then put in distilled water for three days. When the roots were about 2 cm long, the bulbs were transferred to beakers with 5% w/v suspensions of topsoil powders for 48 and 72 h (Grant, 1982; Fiskesjö, 1985). For the control, the roots were incubated in distilled water or suspensions of houseplant soil. The trials were performed in the growth chamber at a temperature of 24 ± 2 °C under white light with a light intensity of 90 $\mu E/m^2\ s^{-1}$ and a light/dark photo-period of 14/10 h. The root length was measured at the beginning and at the end of the treatments. Further, the terminal part of the roots was utilized for cell analysis as described by Yuet Ping et al. (2012). For each sample, several slides were prepared, enabling the observation of at least 1000 cells per sample, and each test was repeated three times. For each preparation, the dividing cells and mitotic abnormalities were counted. The Aberration Index (AI) was calculated as number of aberrations/number of observed cells. The slides were observed using an optical microscope (DMLS, Leica, Wetzlar, Germany), with video camera True Chrome HDII TESSELAB, at 400x magnification.

2.8. Statistical analysis

Statistical analyses were carried out using SPSS Statistics v20. Normality of the data groups were checked by Shapiro-Wilk test. Parametric and nonparametric Levene's tests were applied to check the homogeneity of variances. Differences between the samples and the control groups were determined by ANOVA (Tamhane's T2, Tukey-HSD). Correlations between the measured total PAHs, REEs, metals and C10–C40 concentrations at each site and the results of each biotest were analized by Pearson and Spearman correlation tests. Differences were considered significant when $p < 0.05$. Before analysis, concentrations reported as being below the detection limit (BDL) were recoded to half of the detection limit. The PAH dibenz(a,h)anthracene was removed from the analysis as all values were BDL.

Statistical analyses included a combination of descriptive statistics, correlation analyses and principal components analysis (PCA) to summarize the complex chemical composition of the soil samples using prcomp in R 4.1.2 (R Core Team, 2021). The data were scaled and centered to remove any effects that might result from variable magnitude. Two different PCAs were conducted; the first to explore the variance of the soil contaminants (by only including analized contaminants), and then to explore the variance in assay and soil contaminants data (including all variables).

Due to the low ratio of dependent (assay data) to independent (soil contaminant data), the PCA was also used for dimensionality reduction. Thus, these principal components were used in a regression where fertilization rate, MPE, and development defects were the dependent variables, and the principal components (PC) were the independent variables. The relevant PCs were selected using a backwards stepwise method. To obtain the beta estimates coefficients from the principal component regression, the regression coefficients was multiplied by the contaminants' matrix. This method was solely exploratory and did not have associated p-values for each contaminant.

3. Results

3.1. Organic analyses

Total PAHs and C10–C40 hydrocarbons were found at different concentrations according to the sampling sites, as shown in Table 3. PAHs displayed the highest concentrations at sites #8 and 12, while lesser though still significant concentrations were found at sites #1–2 and #4–6 at Augusta, and site #11–13 at Priolo. PAHs were not found to represent a significant environmental load at sites #14–15. Total C10–C40 hydrocarbons were present at the highest concentrations at sites #3 and 13, with lesser concentrations noted at the other sites.

3.2. Inorganic analyses

Total metal concentrations failed to show any major differences among the soil sampling sites, except for lower concentrations (<1000 mg/kg) found at sites #5 and 9 in Augusta (Table 4).

Unlike total metal concentrations, REEs were found to show major differences in topographic distribution, with the highest concentrations found at sites #4, 7 and 8 in Augusta, and at sites #11, 14 and 15 in Priolo, where REE concentrations in the range of approximately 20–40 mg/kg were noted. These values were 2- to 5-fold higher than the highest REE levels found at industrial facilities in Gardanne and in Taranto (Oral et al., 2019; Trifuoggi et al., 2019), where maximum REE levels were measured at 10 and 5 mg/kg, respectively. Lower, yet relevant REE levels were found at other Augusta sites (14–16 mg/kg; sites #3, 5, 6 and 10). The lowest REE levels were found at sites #1 and 9 in Augusta (an old city gate and a public garden), and at sites #12 and 13 in Priolo.

3.3. Sea urchin bioassays

When *S. granularis* embryos were reared in topsoil-contaminated (0.1% w/v) seawater, they underwent a significant increase in developmental defects *vs.* controls, as shown in Table 5, without any significant distinctions between sampling sites.

By exposing sea urchin sperm to topsoil-contaminated seawater (0.5% w/v), two endpoints were evaluated: percent fertilization success (fertilization rate, FR); and developmental defects in the offspring of topsoil-exposed sperm. As shown in Table 6, most of the sites displayed significant spermiotoxicity at sites # 2 to 5, 7, 9, 12 and 13 ($p < 0.01$) but not at sites # 6, 8 (Augusta), # 11 and 14 (Priolo). The induction of developmental defects in the offspring of topsoil exposed sperm was significantly increased for all the sampling sites, as was consistently observed in three repeated assays ($p < 0.01$ to $p < 0.001$).

Topsoil-exposed embryos were further submitted to cytogenetic analysis, by testing both inhibition, if any, of mitotic activity and of frequency of mitotic animalies, measured as mean number of mitoses per embryo (MPE) and as percent embryos with mitotic abnormalities (%Ab+).

As shown in Table 7, the MPE data showed a highly significant decrease at site #11, and significantly decreased MPE at sites #1, 2 and 4 to 7, whereas the other sites failed to display any significant alteration of MPE compared to controls. By scoring the frequencies of mitotic abnormalities, the %Ab + displayed highly significant increases at sites #1 to 5, 7 and 10 (Augusta). Significantly increased % Ab+ was also found for soil collected at sites #12, 13 and 15 (Priolo), whereas the topsoil from the other sites failed to display any significant increase in % Ab+.

Table 3
Total concentrations of organic contaminants (PAH and C10–C40 hydrocarbons) in Augusta-Priolo soil samples (mg/kg).

Sample #	PAH	C10–C40
Augusta		
1	0.186	145
2	0.167	154
3	0	500
4	0.221	255
5	0.155	352
6	0.128	176
7	0.019	216
8	1.118	85.6
9	0.033	316
10	0	ND
Priolo		
11	0.282	212
12	0.614	155
13	0.333	789
14	0	196
15	0	246

Table 4
Total concentrations of metals and REEs in Augusta-Priolo soil samples (mg/kg).

Sample #	Metals	REEs
Augusta		
1	1323.28	7.45
2	1055.96	ND
3	1238.55	16.61
4	3218.81	38.68
5	941.36	9.416
6	1132.42	14.05
7	1564.08	18.00
8	1426.09	24.34
9	685.44	7.83
10	1052.49	16.16
Priolo		
11	1602.97	18.31
12	1249.80	5.50
13	1724.62	5.60
14	2224.76	18.20
15	1097.12	19.67

Table 5
Percent developmental defects in *S. granularis* embryos/larvae reared in topsoil samples (0.1% v/w) from Augusta-Priolo.

Control	9.33 ± 2.13
#Sites	
Augusta	
1	46.33 ± 8.27
2	51.22 ± 10.52
3	88.11 ± 4.36
4	59.67 ± 10.59
5	67.56 ± 15.45
6	37.89 ± 7.25
7	43.22 ± 3.93
8	22.11 ± 4.46
9	54.22 ± 7.53
10	19.56 ± 4.00
Priolo	
11	33.33 ± 4.92
12	61.89 ± 4.00
13	41.78 ± 6.00
14	39.00 ± 6.15
15	51.22 ± 10.83

Table 6
Percent fertilization (Fertilization Rate, FR) and percent developmental defects (DD) in offspring following 10 min exposure of *S. granularis* sperm to 0.5% (w/v) topsoil.

# Site	FR	DD
Control	78.47 ± 3.23	39.28 ± 3.08
Augusta		
1	66.0 ± 4.34	75.75 ± 6.57***
2	22.92 ± 2.21***	94.67 ± 3.96***
3	39.50 ± 7.10***	93.67 ± 5.94***
4	23.33 ± 5.77***	97.00 ± 10.54***
5	58.75 ± 7.90**	84.17 ± 5.13***
6	68.42 ± 7.70	85.50 ± 6.93***
7	48.00 ± 6.95***	86.50 ± 5.80***
8	72.58 ± 7.10	82.67 ± 4.64***
9	44.83 ± 7.59***	85.20 ± 8.40***
10	60.92 ± 7.83*	97.00 ± 8.93***
Priolo		
11	64.75 ± 9.40	89.33 ± 4.40***
12	40.83 ± 7.94***	81.00 ± 6.88***
13	50.25 ± 7.12***	68.33 ± 7.25***
14	80.75 ± 4.12	59.33 ± 5.79**
15	62.00 ± 5.05*	88.17 ± 5.13***

Table 7
Mean number of mitoses per embryo (MPE) and % embryos with ≥1 aberrations (% Ab+) in *S. granularis* embryos exposed to topsoil 0.1% w/v and fixed 5 h p-f.*: $p < 0.05$, *: $p < 0.01$,***: $p < 0.001$ (ANOVA LSD).

# Site	MPE	% Ab+
Blank	10.02 ± 2.09	14.97 ± 2.8
Augusta		
1	4.57 ± 1.80 *	55.30 ± 6.20 **
2	4.50 ± 1.01 *	66.10 ± 8.20 ***
3	4.97 ± 1.34	62.30 ± 4.29 ***
4	3.03 ± 1.07 *	58.90 ± 4.08 **
5	3.50 ± 0.56 *	56.13 ± 15.29 **
6	3.40 ± 1.56 *	36.57 ± 1.68
7	3.30 ± 0.79 *	43.27 ± 9.32 *
8	5.70 ± 1.10	31.97 ± 0.67
9	4.70 ± 1.61	37.80 ± 20.30
10	4.97 ± 1.57	56.73 ± 7.40 **
Priolo		
11	1.83 ± 0.66 **	33.80 ± 12.44
12	7.87 ± 3.64	38.50 ± 16.69 *
13	11.10 ± 2.46	45.10 ± 14.28 *
14	6.50 ± 1.65	38.37 ± 11.27
15	8.03 ± 2.75	44.93 ± 11.13 *

3.4. Bulb onion assays

No significant changes in mitotic activity were detected in *A. cepa* root tips exposed to 5% topsoil suspensions (data not shown). However, the induction of mitotic aberrations, calculated as number of aberrations/number of observed cells, aberration index (AI), displayed some differences in-between topsoil sampling sites, after 72 h of treatments. Sites # 5 and 14 showed highly significant AI excess after 72 h of treatment, with lesser, yet significant AI increase at sites # 6, 10 and 13. No significant increase in AI was found at the other 10 site and after 48 h of treatments (Table 8).

3.5. Microalgae assays

By rearing *P. tricornutum* microalgae for 72 h in topsoil-contaminated medium (1:4 w/v), spectrophotometric measurement of samples showed highly significant cell growth inhibition for sites # 2, 6, 8, 9, 11 and 15, as shown in Table 9. Lesser, yet significant growth inhibition was found for sites # 1 and 12, while no significant growth inhibition was found for the other sites (# 3, 4, 7, 13 and 14).

3.6. Nematode assays

Topsoil-exposed *C. elegans* displayed different mortality rates. The only site displaying highly significant mortality was # 9, while other sites displayed lesser, yet significant *C. elegans* mortality, namely # 1, 3, 4, 7, 11, and 12 (Table 10). No significant effects were found for topsoil from the other sites (# 2, 5, 6, 8, 10, 13 and 14).

3.7. Summarizing the results of the different bioassays

The overall results of the different bioassays testing topsoil and dust toxicity at Augusta-Priolo sampling sites are summarized in Fig. 2. Highly significant adverse effects ($p < 0.01$) were consistently found at sites # 2, 3, 4, and 5 across the different bioassays. Lesser, yet significant effects ($p < 0.05$) were found at sites # 6, 7, 9, 10, 11 and 14, whereas no significant adverse effects were observed at the other sites (# 1, 8, 12, 13 and 15).

3.8. Spatial patterns in soil contaminants and sea urchin bioassay responses

We carried out regression analysis of the fertilization rate, mitoses per embryo and developmental defects percentage in the sea urchin

Table 8
Percent of mitotic aberrations (Aberration Index, AI) in onion roots treated for 48 and 72 h with 5% suspensions of topsoil samples from Augusta and Priolo sites.Values represent the mean (±SD) of three experiments. **values that are statistically different $p \geq 0.001$; * statistically different $\geq$ p 0.05%.

# Site	AI 48 h	AI 72 h
Control	1.01 ± 0.12	1.05 ± 0.08
Augusta 1	2.52 ± 2.48	0.35 ± 0.04
2	0.01 ± 0.01	0.40 ± 0.36
3	0.40 ± 0.30	0.20 ± 0.12
4	0.47 ± 0.29	0.44 ± 0.50
5	0.06 ± 0.10	4.80 ± 0.50**
6	0.28 ± 0.03	1.90 ± 0.20*
7	0.17 ± 0.02	0.26 ± 0.03
8	0.40 ± 0.30	0.31 ± 0.30
9	0.10 ± 0.05	0.12 ± 0.09
10	0.20 ± 0.01	1.21 ± 0.10*
Priolo		
11	0.20 ± 0.01	0.32 ± 0.03
12	0.73 ± 0.02	1.20 ± 0.03
13	0.15 ± 0.01	3.51 ± 0.03*
14	0.17 ± 0.04	12.05 ± 0.02**

Table 9
Algal growth inhibition (%) in *P. tricornutum* exposed to topsoil 1:4 w/v.*: $p < 0.05$, **: $p < 0.01$,***: $p < 0.001$ (t-Student test).

#Site	Algal growth inhibition (%)
Augusta	
1	−0.27 ± 1.55
2	10.76 ± 2.33**
3	15.45 ± 2.68**
4	−0.94 ± 0.92
5	−11.89 ± 0.58***
6	9.86 ± 0.4 ***
7	19.88 ± 1.59***
8	−2.51 ± 1.18
9	41.19 ± 1.51***
10	13.59 ± 1.19***
Priolo	
11	9.69 ± 1.24**
12	15.01 ± 4.77**
13	0.098 ± 0.31*
14	−4.59 ± 3.25
15	−0.27 ± 1.55

Table 10
Mortality (%) in *C. elegans* exposed to Augusta and Priolo topsoil.*: $p < 0.05$, **: $p < 0.01$,***: $p < 0.001$ (t- Student test).

#Site	Mortality (%)
Augusta	
1	23.33 ± 3.33**
2	16.66 ± 3.33
3	36.66 ± 3.33**
4	26.66 ± 6.67
5	46.66 ± 6.76**
6	26.66 ± 6.67
7	46.66 ± 6.76**
8	6.66 ± 3.33
9	56.66 ± 3.33***
10	16.66 ± 3.3
Priolo	
11	26.66 ± 3.33**
12	43.33 ± 3.33**
13	3.33 ± 3.33
14	3.33 ± 3.33
15	26.66 ± 6.76

assays by distance and direction from the oil refinery. The distance to the refinery was not predictive of the assay results, and the direction of the sampling sites with respect to the oil refinery was only significant for MPE. MPE was found to be the only endpoint with a distinctive pattern, where higher rates of MPE were southeast of the oil refineries. An analysis of predominant winds at the study sites show them to be primarily from the SE, blowing NNW (Fig. 1S) (https://www.meteoblue.com/it/tempo/mappa/precipitazione/italy). This might suggest airborne deposition of particulates carrying some of these pollutants in topsoil northwest of the refineries.

The results of the PCA revealed that, together, the first four components explain 78.6% of the variance. In Fig. 3A, the contaminant groups (metals and REEs, and PAHs) cluster together. Most of the variance in this plot appears to be driven by a few sites, notable site # 8, associated with PAHs, and site # 4, associated with metals and REEs. Fig. 3C shows no distinctive pattern in the variance structure of contaminants between site locations. Fig. 3B and D, encompassing the third and fourth PCA components and explaining less of the variance, show how the location of many of the contaminants is more mixed than that given in the first two PCA components. The chemical composition of mainly PAHs and metals are associated with sites # 11–14, whereas sites # 6, 7 and 10 are associated with the REEs. There is also a distinctive pattern in the variance structure of contaminants between site locations. The sites associated with metals and PAH are distinctive from sites associated with REEs.

In Fig. 4A and B comparing the results of the sea urchin bioassay data with soil contaminants, the fertilization rates and developmental defects are directly opposed to each other in all of the biplots. The fertilization rates cluster with As and some PAHs. These assay and metals are associated with sites # 6, 8, and 10 in Fig. 3A and with sites # 6, 11, and 14 in Fig. 3B. MPE clusters with some PACs and some metals in both 4 A and 4 B. These contaminants and assays are associated with sites # 9,12 and 13 in Fig. 3A and sites # 12 and 13 in Fig. 3B. In Fig. 3A, developmental defects cluster with metals including Hg, Se, and B while they are associated with metals and PAHs in 4 B. These patterns in assays and contaminants are associated with sites # 3 and 15 in Fig. 3A and sites # 4, 8, and 12 in Fig. 3B.

The principal components from Fig. 3A and B were used in a principal components linear regression model to estimate the beta coefficients of the dependent variables based on the PCA. The fertilization rate was best explained by PC2 and PC4 (Adj. $R^2 = 0.31$, $p = 0.04$). The degree of developmental defects was best explained by PC4 (Adj. $R^2 = 0.17$, $p = 0.065$) while MPE was best explained by PC3 (Adj. $R^2 = 0.14$, $p = 0.093$). For the fertilization rate and rate of developmental defects, many contaminants are negatively associated with these dependent variables, whereas many contaminants are positively associated with MPE. Metals Zn, Sb, Cu, Sn, Hg had the largest negative effect on the fertilization rate. Cadmium, and the PAHs Phenanthrene, Fluorene, Acenaphthene, had a strong positive association with MPE. In contrast, PAHs Acenaphthylene and Naphthalene had a negative relationship with developmental defects, while the positive beta coefficients of Hg and Zn indicate a protective effect.

4. Discussion

An extensive body of literature has investigated topsoil pollution and toxicity in a number of urban, suburban and industrial locations (e.g. Li et al., 2015; Rinklebe et al., 2019; Luo et al., 2020). We have previously reported on topsoil pollution and toxicity close to bauxite processing plants in Gardanne (southern France) and Portovesme (Sardinia, Italy) (Pagano et al., 2002; Oral et al., 2019), and close to a steel foundry in Taranto (Italy) (Trifuoggi et al., 2019). Those studies allowed us to find topographic associations of topsoil toxicity with contaminant levels and directions of prevailing wind regimens.

Moreover, chemical mixtures of environmental pollutants in the vicinity of petrochemical industries have become the focus of much current interest, with studies recently reporting on characterization of these mixtures (Signa et al., 2017; Relić et al., 2019; Di Bella et al., 2020). The presence of such classes of compounds have also for some time raised concerns about potential impacts on human and environmental health (Park et al., 2006; Weng et al., 2008; Cernigliaro et al., 2016, 2019; Reinecke et al., 2016; Santoro et al., 2017; Yuan et al., 2018; Kafaei et al., 2019). In particular, topsoil from areas around oil refineries have been the subject of both chemical analyses (Li et al., 2009; Di Leonardo et al., 2014; Bayat et al., 2015; Benlaribi and Djebbar, 2020; Di Bella et al., 2020; Wang et al., 2020) and biological effects assessments using specific bioassay models which have provided evidence for multiple adverse effects (Höss et al., 2009; González et al., 2013; Hentati et al., 2013; Reinecke et al., 2016; Maisano et al., 2017; Copat et al., 2020).

In the present study, the use of multiple bioassays coupled with a robust chemical analysis of topsoil chemical mixtures allowed the modeling of dose-response relationships of exposure to these mixtures at environmentally relevant concentrations through multivariate analyses. In particular, it was found that the most polluted sites, for example at the Punta Cugno facilities (sites # 3–5), displayed the highest adverse effects, unlike relatively unpolluted sites, such as # 10–15 located in town suburbs or at an archeological site. Indeed, these spatial patterns were confirmed by both bioassays and chemical analyses, and uncovered the likely influence of wind patterns on particulate deposition away from

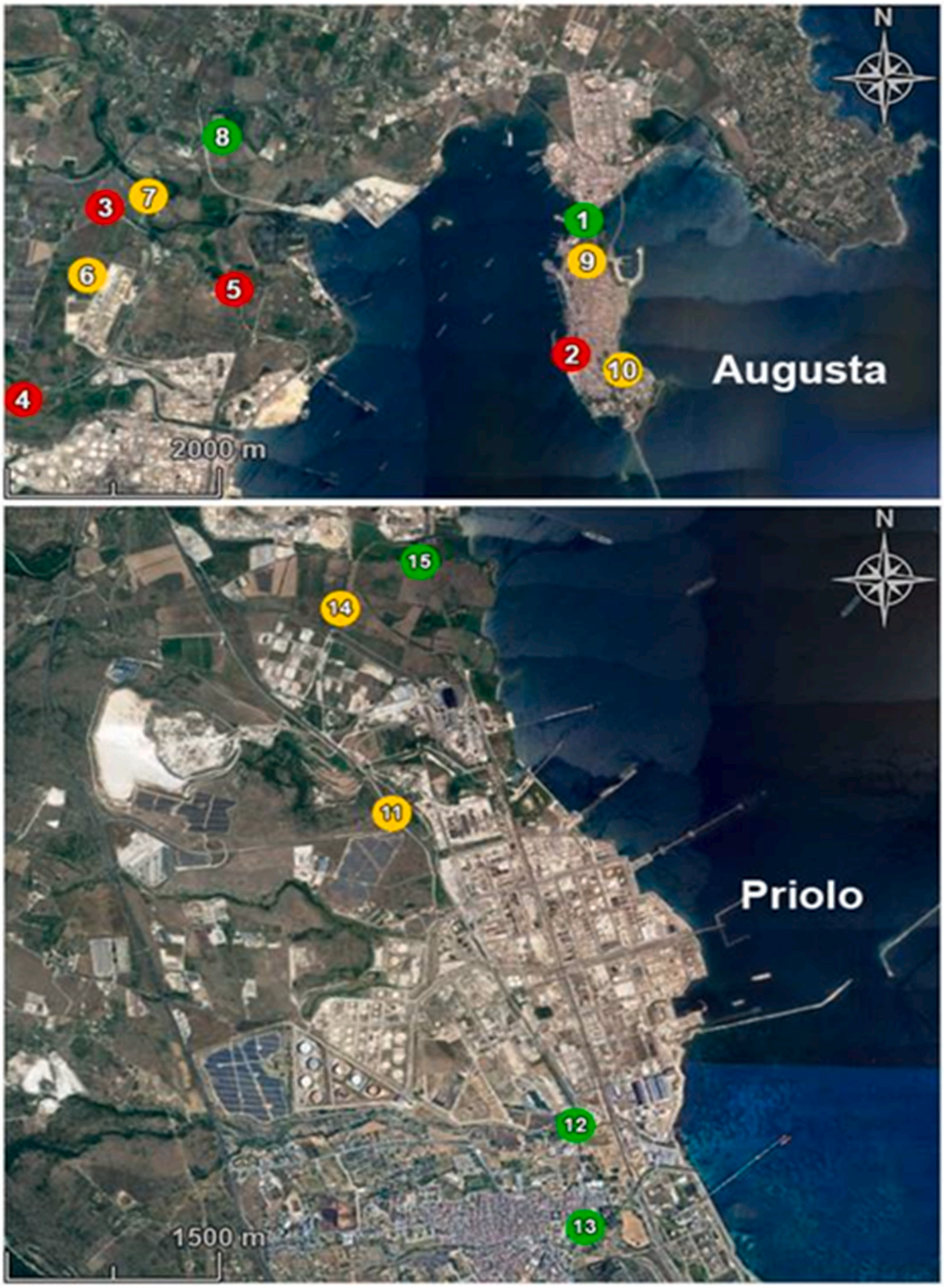

Fig. 2. Summarized bioassay data from sea urchins (*S. granularis*), diatoms (*P. tricornutum*), nematodes (*C. elegans)*; and onions (*A. cepa)*. Site colors are attributed as green ($p > 0.05$), yellow ($p < 0.05$) and red ($p < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the town towards the northwest. Less polluted sites were thus located for the most part in areas with higher population numbers. Further, chemical analyses revealed elevated hydrocarbon and metal concentrations in closer proximity to sites with higher petrochemical activities and supporting industry. Considering the significantly elevated incidence of congenital anomalies such as genital and heart defects in live births in the region, for example a 2.5 times higher incidence (over national baseline values) of male hypospadias in newborns in the 1991–2002 period (Bianchi et al., 2006), the results reported herein can support epidemiological risk analysis that encompasses factors such as medical data and geospatial proximity to the industrial facilities.

An important finding was that the study uncovered a spatial patterns of REEs in topsoil samples. Higher environmental loadings of REEs were detected in topsoils of this study when compared to other similar studies (Pagano et al., 2002; Oral et al., 2019; Trifuoggi et al., 2019). These results might confirm the ability to use REE patterns as a fingerprint in identifying likely sources of environmental pollutants as REEs are often used in oil refining processes (e.g. La) and as catalytic additives (Ce) to diesel oil (Cassee et al., 2011; Dale et al., 2017; Kumar et al., 2019). From a human health perspective, whether such elevated REE levels should be a cause for concern is not yet clear as there are still many knowledge gaps on REE toxicity. Given the necessary use of REEs in oil

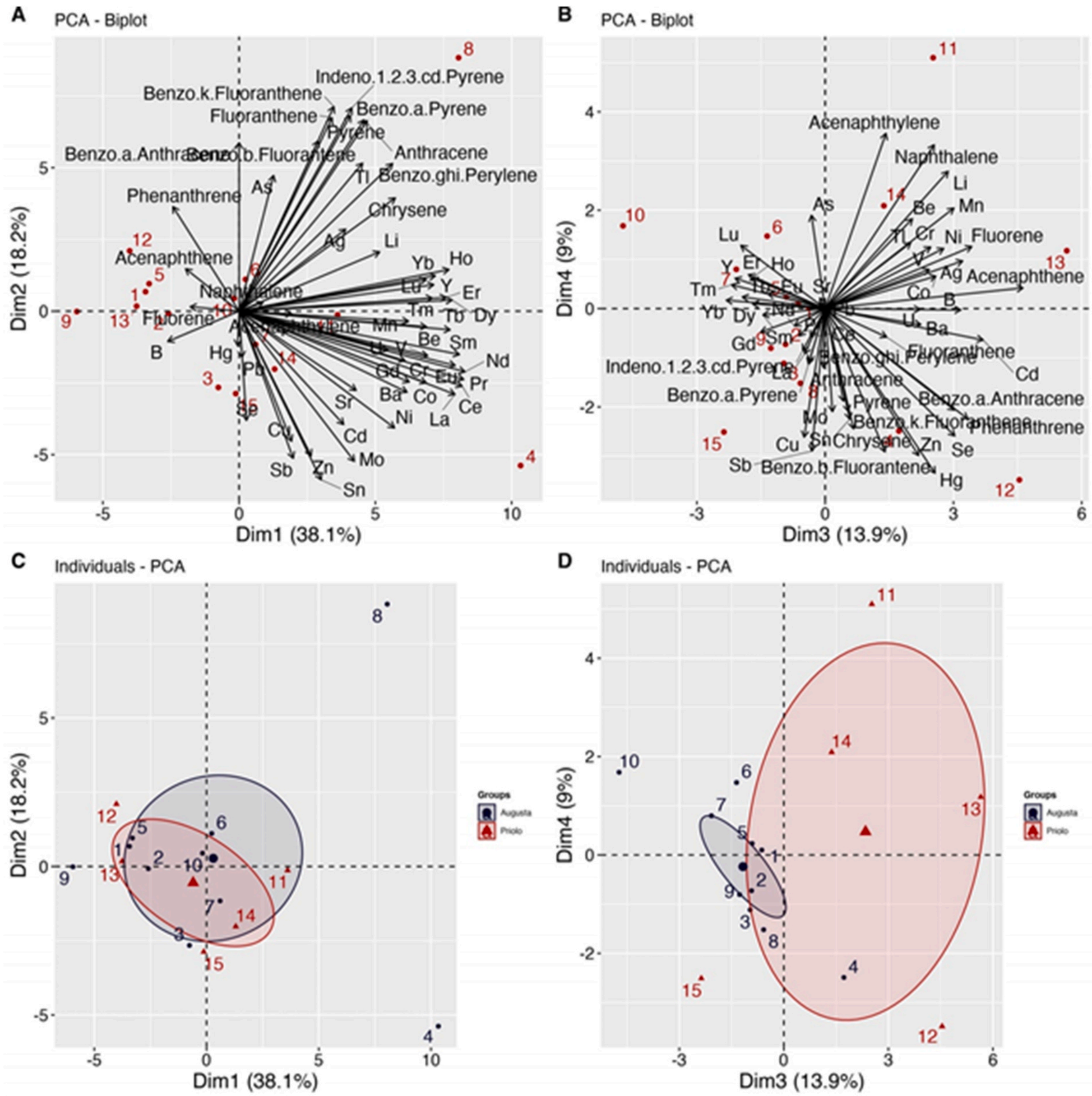

Fig. 3. Principal components analysis (PCA) biplots for the environmental contaminants in topsoil for (A) PC1-2, and (B) PC3-PC4. PCA-related site patterns are given in (C) and (D) The ellipse indicates the 95% confidence interval of the cluster.

refining and in the production of diesel catalytic additives, further investigations may be warranted on occupational exposures to oil products and exhaust microparticulate matter in this region (Snow et al., 2014; Pagano et al., 2019).

5. Conclusions

The present study provides a geospatial assessment of multiple environmental contaminants present as complex mixtures in topsoils collected in an industrialized region near Augusta and Priolo (SE Sicily). By including both biological effect assessments and contaminant analyses in these multivariate analyses, spatially-explicit dose-response relationships may be uncovered to reveal likely sources of pollutants, and could be used to evaluate human health risk assessments and to develop potential mitigation strategies through engagement with epidemiologists and local government administration. Some of the more prominent and heavily polluted sampling sites were identified with associated biological effects in a panel of model organisms. These sites were located close to some defined petrochemical facilities as, for example, the "Punta Cugno" site (# 3–5). A noteworthy finding was the increased level of total REEs compared to other industrial facilities investigated previously. This observation was consistent with the recognized roles of REEs both in oil refining and in the utilization of catalyic additives in the formulation of diesel fuel. In turn, this finding is in agreement with the occupational REE exposure in workers exposed to diesel exhaust microparticulate matter.

Author contribution statement

Franca Tommasi: Conceptualisation and planning; Giovanni Pagano and Daniel M. Lyons: Writing Original Draft; Philippe J. Thomas: Data curation, Review and Editing; Serkan Tez and Kristin M. Eccles: Software, Visualisation; Rahime Oral, Antonietta Siciliano, Isidora Gjata, Andrej Jaklin, Petra Burić, and Ines Kovačić: Bioassay performing; Maria Toscanesi and Antonella Giarra: Inorganic and organic analyses; Marco Guida and Giovanni Libralato: Data curation, Writing; Marco Trifuoggi:

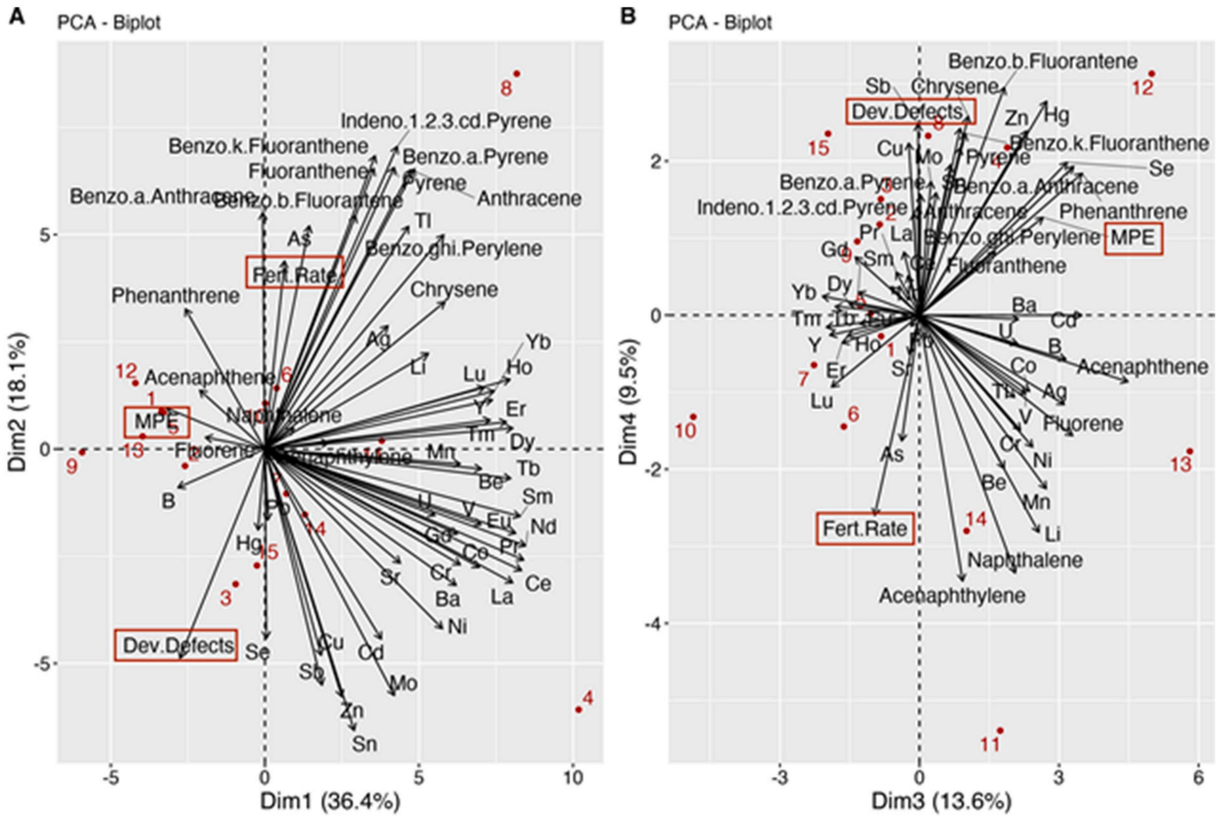

Fig. 4. Principal components analysis biplots for the environmental contaminants in soil with assay data for (A) PC1-2, and (B) PC3-PC4. The sea urchin bioassays are highlighted with a red box. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

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Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.138802.

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