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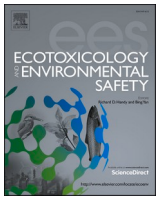

Exposure to multiple metals/metalloids and human semen quality: A cross-sectional study

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ABSTRACT

Background: Exposure to metals/metalloids, including essential and nonessential elements, has been associated to male reproductive health in animals. However, findings from human studies are inconsistent.
Objectives: To investigate the impact of exposure to multiple metals/metalloids at environmental levels on the conventional human semen-quality parameters.
Materials and methods: Men living in rural or industrial areas were recruited by personalized letters. No exclusion criteria were applied. Each man provided one semen sample and one blood sample. We analyzed the semen sample both to determine conventional sperm parameters (concentration, progressive motility and normal forms) and to quantify lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), nickel (Ni), vanadium (V) and selenium (Se) levels. The levels of these metals/metalloids were also quantified in venous blood and spermatozoa samples. Associations between the blood/seminal plasma metal/metalloid levels and semen quality parameters were assessed using confounder adjusted logistic regression models. Correlation and interactions between blood/seminal plasma and semen metal/metalloid levels were investigated using the Spearman's correlation.
Results: We found a positive association of seminal plasma cadmium level with lower Total count (OR = 4.48, 95%CI 0.25–80); whereas lead (OR = 4.51, 95%CI 0.86–23) and cadmium (OR = 3.45, 95%CI 0.77–16) seminal plasma levels had a positive association with progressive sperm motility. Overall, these associations remained suggestive after adjustment, though statistically unstable risks. Finally, we found weak interactions between beneficial effects of Se and detrimental ones only for Cd and Pb blood level on sperm concentration, total sperm count and progressive sperm motility.
Conclusions: Our findings suggest that environmental exposure to Pb and Cd contributes to a decline in human semen quality, whereas Se can have beneficial effects. Measurements of metals/metalloids in the seminal fluid may be more predictable of semen quality than conventional blood measurements

1. Introduction

In the last decades, human fertility appears to be declining and the male factor is estimated to contribute by about 15–20% of the infertility cases (Choy and Eisenberg, 2018). Many studies suggest that a worsening of the semen quality is one of the main contributing factors (Carlsen et al., 1992; Templeton, 1995; Itoh et al., 2001; Danadevi et al., 2003; Sokol et al., 2006; Swan, 2006; Murawski et al., 2007; Stewart et al., 2009).

Several factors can influence sperm parameters, such as age, genetic background, environmental, occupational, and lifestyle factors (Jurewiez et al., 2014; Knez, 2013; Bonde, 2013; Sharma et al., 2013). Increasing evidence suggests that occupational and environmental exposure to toxic pollutants may contribute to the decline of male fertility (Mima et al., 2018; Zhou et al., 2019), though conflicting data have been reported (Saidi et al., 1999; Fisch, 2008). A study on current sperm count trends reported a significant 50–60% fall in total sperm count among men in North America, Europe, Australia, and New

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Zealand (Levine et al., 2017). Furthermore, the harmful impact on the male reproductive system of some lifestyle-related factors, such as cigarette smoking, alcohol intake, drug abuse, obesity, and psychological stress, could intensify from one generation to another (transgenerational effect) (Soubry et al., 2014). However, it is difficult to assess the burden that each of these factors has on male fertility, since often exposure to these risk factors does not occur individually but rather simultaneously, with different duration and intensity of exposure for each of them (Levine et al., 2017). In-vitro studies have shown the detrimental effect of cigarette smoke compounds on sperm parameters (Condorelli et al., 2013; Alamo et al., 2019). Exposure to environmental pollutants, such as petroleum products, agrochemicals, industrial chemicals, and heavy metals, especially lead (Pb) and cadmium (Cd), by contaminating air, food, and water, can also negatively interfere with the male reproductive system (Wong and Cheng, 2011; Wijesekara et al., 2015). Moreover, certain essential (e.g., Se) and non-essential elements (e.g., Cd and Pb) may promote an additive, synergistic or antagonistic damage on the reproductive function (Meeker et al., 2008).

According to these observations, the present study aimed to investigate the impact of exposure to multiple metal/metalloid elements [Lead (Pb), cadmium (Cd), selenium (Se), mercury (Hg), arsenic (As), nickel (Ni), and vanadium (V)] at environmental level on the conventional human semen-quality parameters.

2. Materials and methods

The study was conducted with a cross-sectional design. The institutional ethical committee of the University Teaching Hospital "G. Rodolico" (Catania 1) approved the protocol and all eligible participants signed an informed consent form prior to enrollment.

2.1. Study population

The recruitment was carried out in two different areas of eastern Sicily (South Italy): the first in an industrial area (Melilli, Siracusa) and the second in an agricultural one (Regalbuto, Enna), so we recruited men potentially subject to both environmental and occupational exposure to metals/metalloids. They were contacted from the general population by sending a personalized letter and those who accepted were enrolled and underwent andrological visit, as established by the experimental protocol, without exclusion criteria. None of the subjects were already admitted to our Endocrinological department for pre-conception consultation or had been experienced some conception issues. From March 2017 to October 2019, a total of 400 men were invited to participate in the study, of which 179 (acceptance rate 44.7%) ultimately enrolled. Each participant was asked to complete a face-to-face questionnaire on the day of his clinical visit. The information collected included demographic characteristics, habits and lifestyle, occupational exposure and clinical data. Moreover, each men participating to the study underwent to an accurate anamnesis, physical examination, semen collection, and blood withdrawal in the same day.

2.2. Seminal fluid and blood collection analyses

Seminal fluid was collected after 3–5 days of abstinence; each participant provided a semen sample into a sterile wide-mouth metal-free polypropylene container.

After liquefaction, it was analyzed according to the World Health Organization criteria (WHO, 2010) to evaluate conventional sperm parameters (concentration, progressive motility, and normal forms). The semen volume (mL) was measured using a metal-free polypropylene gradu- ated pipettes.

One aliquot of seminal plasma and one of seminal pellet were frozen at − 20 °C until the time of analysis. It was digested with 65% nitric acid (HNO_3) (Fisher Scientific, USA) and 30% hydrogen peroxide (H_2O_2), filtered, and diluted up to 5 mL to quantify the levels of Pb, Cd, Hg, As, Ni, V, and Se.

Venous blood samples were collected, using stainless-steel needles, into plastic tubes certified metal free and stored at − 20 °C. Whole blood samples (1 mL) were digested with 65% nitric acid (HNO_3) and 30% oxygen peroxide (H_2O_2), filtered and diluted up to 10 mL to quantify the levels of Pb, Cd, Hg, As, Ni, V, and Se.

All samples were digested in a closed microwave system (temperature program reaching 120 °C that was maintained for 10 min at 1000 W) equipped with Teflon vessel [Ethos TC, Milestone, Sorisole (BG), Italy]. Double distilled purified deionized water from Milli-Q system was used for dilution before the analyses. All glassware was washed and immersed in concentrated HNO_3 overnight and then rinsed with deionized water before use.

We have quantified metals/metalloids by Inductively Coupled Plasma Mass Spectrometry (ICP-MS Elan DRC-e, Perkin Elmer - Mundelein, Illinois), except for total Hg that was quantified using FIAS 100 system coupled with an Atomic Adsorption Spectroscopy (AAS) (AAnalyst800, Perkin Elmer, LabX, Midland, Canada).

To guarantee the accuracy and precision of these measurements, an appropriate control was added for each element. To accomplish this, certified reference material Seronorm™ Trace Elements Whole Blood (Billingstad, Norway) was used for each batch of analysis.

We calculated the minimum level of substance that can be measured and reported with 99% confidence that the analyte level is greater than zero (Table 1).

In particular, the limit of detection (LOD) estimated with processed blanks were calculated according to the following equation:

$$LOD = X + 3\sigma$$

Where X is the mean of processed blanks and σ its standard deviation (Armbruster and Pry, 2008).

2.3. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Science (SPSS), version 21.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics [mean, median, standard deviation (SD) and interquartile range (IQR, 25th–75th), proportion (%)] were used to describe the study population demographic, lifestyle and clinical characteristics, distributions of blood/seminal fluid metals/metalloids and semen quality outcome measures.

Metal/metalloid levels below Method Detection Limits (MDL) were replaced by MDL/2 for the statistical elaboration.

We compared the distribution of demographic, lifestyle and clinical characteristics and metal/metalloid levels both by residence (rural area and industrial one) and sperm quality (NSP group: men with normal sperm parameter; ASP group: men with ≥ 1 abnormal sperm parameter). In particular, the study participants were classified as either being below or at/above the WHO reference levels for their progressive motility (32% motile sperm), total motility (40% motile sperm), concentration (15 million/mL) and total count (39 million). Participants with all four parameters at/above the reference levels were categorized as the comparison group (NSP). Moreover, distribution of blood and semen metal/

Table 1
Limit of detection (LOD) of metals/metalloids, by blood, seminal plasma and spermatozoa.

	Blood (μg/L)	Seminal plasma (μg/L)	Spermatozoa (μg/g)
Arsenic	0.050	0.031	0.084
Cadmium	0.073	0.007	0.157
Lead	1.133	0.038	0.183
Mercury	0.103	0.098	0.456
Nichel	5.505	0.214	1.086
Selenium	0.102	0.095	0.145
Vanadium	0.778	0.057	0.171

metalloid levels stratified by smoke habits was also performed.

Spearman's correlation analysis was used to investigate the correlation between blood and semen metal/metalloid levels; the strength of linear relationship corresponding to the correlation coefficient value was evaluated according to Chan YH (Chan, 2003).

Furthermore we explored the possible interactions between beneficial effects of selenium and detrimental ones of the others metal/metalloid investigated.

Potential confounder factors were included in the multivariate models based on statistical considerations. The confounding factors were chosen through verification during the analysis of their confounding effect. Therefore, we considered confounding the variables that led to a change in the estimate of the association between exposure and outcome greater than 20–30% (Hennekens and Buring, 1987).

Logistic regression models were used to assess the relationship between seminal plasma metal/metalloid levels and conventional sperm parameters. Sperm parameters were dichotomized as below at or above the WHO reference values (sperm concentration > 15 million/mL, total sperm count >39 million/ejaculate, progressive sperm motility > 32%) (WHO, 2010). Results were expressed as odds ratios (ORs) with 95% CI of sperm parameters in the three seminal plasma element categories (< 50th, 50–75th and > 75th), using the 50th as a referent category.

3. Results

We recruited 179 men, with a mean age (± SD) of 32 ± 6 years (18–46 years). According to the WHO criteria (WHO, 2010), 48 (26.8%) had normal sperm parameters and were considered as the comparison group. In contrast, 21 (11.7%), 14 (7.8%) and 83 (46.4%) men had sperm concentration, total sperm count, and progressive sperm motility, respectively below the reference values. No man enrolled in this study had teratozoospermia. A total of 56 men (31.3%) had more than one abnormal sperm parameter. We did not observe any differences in the median time of liquefaction (40 s vs 40 s), pH (8.1 vs 8.2) and volume (3.4 mL vs 3.0 mL) between men with normal sperm parameters vs abnormal ones.

Demographic, lifestyle, and clinical characteristics, by healthy status and residence area are shown in Tables 2 and S1, respectively.

3.1. Metal/metalloid blood, seminal plasma, sperm levels and semen parameters

Distribution of metal/metalloid blood, seminal plasma, spermatozoa levels and sperm parameters, by NSP and ASP groups are shown in Table 3. ASP group had higher blood As (6.14 vs. 4.08 μg/L) and Ni (9.42 vs. 2.75 μg/L) level than NSP group. Both groups had blood levels of Hg slightly higher than the reference values (RV) (ASP: 5.70 μg/L vs. NSP: 5.28 μg/L). ASP and NSP groups had equal Cd median blood levels, whereas the stratified analysis by smoking habits showed slightly higher median levels in smokers than in non-smokers both belonging to the NSP group (0.31 vs 0.24 μg/L) and to the ASP one (0.48 vs 0.26 μg/L).

ASP group had higher seminal plasma Pb (14.35 vs. 2.83 μg/L) and Cd (0.93 vs. 0.43 μg/L) and lower seminal plasma Se (1.17 vs. 12.36 μg/L) levels than NSP group (Table 3).

The spermatozoon levels of heavy metal did not differ between the two groups, whereas Se level was higher in NPS than APS group (0.219 μg/L vs 0.193 μg/L).

As expected, all sperm parameters of the NSP group were significantly higher than those of ASP group (Table 3).

Distribution of metal/metalloid blood, seminal plasma, spermatozoa levels, by industrial and rural areas are shown in Table S2. Blood levels of Pb and Cd were higher in the blood of men living in the rural area, whereas the blood levels of Se, As, Ni, and V were higher in men living in the industrial area. Hg blood levels did not seem different between the groups of men, whereas seminal plasma level in rural group was higher than in industrial one (Table S2). Men living in the industrial area had higher seminal plasma levels of Pb and Cd compared with men living in the rural area. The levels of Se, Hg and V were higher in men living in the rural area compared with those found in men living in the industrial area. The seminal plasma levels of As and Ni were similar in the two groups of men (Table S2). Finally, Pb and Hg intracellular levels were similar, whereas the intracellular levels of Cd, Se, As, Ni and V were higher in spermatozoa of men living in the rural area compared with those living in the industrial area (Table S2).

Table 2
Demographic, lifestyle and clinical characteristics of men (n = 179), by NSP (men with normal sperm parameter) and ASP (men with > 1 abnormal sperm parameter) groups.

Variables	NSP group[a] n (% column) N = 48	ASP group[b] n (% column) N = 131
Median Age (years) (IQR[c])	32 (26–36)	32 (28–37)
Current smokers	25 (61.0)	48 (39.3)
Cigarette cigarette/day (IQR)	12 (5–20)	10 (4–19)
≤ 5 cigarette/day	6 (25.0)	14 (29.8)
6–18 cigarette/day	10 (41.7)	20 (42.6)
19 + cigarette/day	8 (33.3)	13 (27.7)
Ex-smokers	13 (31.7)	25 (20.5)
Cigarette cigarette/day		
≤ 5 cigarette/day	32 (66.7)	101 (77.1)
6–18 cigarette/day	5 (10.4)	11 (8.4)
19 + cigarette/day	11 (22.9)	19 (14.5)
Never smokers	10 (24.4)	55 (45.2)
Alcohol consumption	18 (43.9)	46 (37.7)
Alcohol consumption glass[d] /day		
≤ 2 glass/day	39 (95.1)	117 (97.5)
3–6 glass/day	1 (2.4)	2 (1.7)
7 + glass/day	1 (2.4)	1 (0.8)
Hashish or marijuana smokers	11 (11.5)	15 (22.4)
Anabolic drugs	0 (0)	2 (1.6)
Industrial workers	9 (22.0)	38 (31.4)
Median BMI (kg/m^2) (IQR)	23.5 (21.1–25.3)	23.7 (21.4–25.9)
Obesity (BMI ≥ 30 kg/m^2)	2 (4.9)	11 (9.0)
Median Meat consumption (times weekly frequency) (IQR)	3 (2–4)	3 (2–5)
Median Fish consumption (times weekly frequency) (IQR)	1 (1–2)	2 (1–2)
Median Fruits and vegetable consumption (times weekly frequency) (IQR)	7 (3–7)	7 (3–7)
Systemic diseases (excluded the subsequent)	21 (51.2)	55 (45.5)
Diabetes	0 (0)	2 (1.6)
Endocrine disorders	10 (24.4)	40 (33.1)
Urogenital abnormalities at the physical examination	23 (59.0)	80 (65.6)
Tumors (any type)	12 (29.3)	25 (35.2)
Cryptorchidism	0 (0)	8 (6.6)
Sexually transmitted diseases	7 (17.1)	17 (13.9)
Head injuries	0 (0)	6 (4.9)
Varicocele	11 (30.6)	15 (16.7)
Hypospadias	0(0)	1 (0.8)
Prostatitis	4 (9.8)	11 (9.0)
Andrological and urological surgery	3 (7.3)	14 (11.5)
Andrological radiotherapy	0 (0)	1 (0.8)
Liver disorders	0 (0)	4 (3.3)
Urogenital infections	1 (2.4)	8 (6.6)
Chemotherapy	0 (0)	2 (1.6)

For dichotomous variables (yes/no), "yes" percentage was reported.
[a] NSP: men with sperm concentration ≥ 15 mil/mL, total sperm count ≥ 39 mil/ejaculate, progressive sperm motility ≥ 32% and normal forms ≥ 4%.
[b] Crude model, 50th as reference exposure category.
[c] IQR: interquartile range in parentheses.
[d] One glass: 330 cc beer, 125 cc wine, 40 cc liqueur.

3.2. Distribution of metals/metalloids in blood, seminal plasma, and spermatozoa, by sperm parameter

Table 4 shows the distribution of metal/metalloid levels in blood, seminal plasma, and spermatozoa by sperm parameters. Pb median

Table 3
Distribution of metal/metalloid levels in blood (B), seminal plasma (SP) and spermatozoa (S) (μg/L and μg/g for spermatozoa) and semen parameters, by NSP (men with normal sperm parameter) and ASP (men with >1 abnormal sperm parameter) groups.

Metals/metalloids	NSP[a] group N = 48 Median (IQR)	ASP[b] group N = 131 Median (IQR)
As (B)	4.08 (2.35–8.55)	6.14 (3.06–9.52)
As (SP)	2.62 (0.40–4.10)	1.78 (0.40–5.06)
As (S)	0.007 (0.003–0.019)	0.007 (0.003–0.020)
Cd (B)	0.32 (0.12–0.96)	0.32 (0.18–0.76)
Cd (SP)	0.43 (0.22–0.85)	0.93 (0.38–1.98)
Cd (S)	0.006 (0.005–0.015)	0.006 (0.004–0.017)
Hg (B)	5.28 (2.40–10.33)	5.70 (3.12–8.90)
Hg (SP)	1.20 (1.20–4.73)	1.20 (1.20–3.25)
Hg (S)	0.014 (0.010–0.022)	0.014 (0.010–0.030)
Ni (B)	2.75 (2.75–11.58)	9.42 (2.75–21.02)
Ni (SP)	2.68 (2.68–11.08)	2.68 (2.68–8.13)
Ni (S)	0.041(0.029–0.100)	0.041 (0.026–0.104)
Pb (B)	12.8 (7.2–18.4)	11.1 (6.8–11.1)
Pb (SP)	2.83 (0.45–14.55)	14.35 (0.45–30.33)
Pb (S)	0.012 (0.007–0.049)	0.016 (0.006–0.040)
Se (B)	115.2 (80.4–130.8)	111.2 (76.3–145.2)
Se (SP)	12.36 (1.18–35)	1.17 (1.18–14)
Se (S)	0.219 (0.138–0.353)	0.193 (0.115–0.387)
V (B)	7.22 (2.70–11.80)	7.05 (0.39–12.13)
V (SP)	4.90 (3.42–17.7)	4.28 (2.10–15.0)
V (S)	0.008 (0.005–0.019)	0.007 (0.005–0.022)
Semen parameters[c]		
Sperm concentration (million/mL)	81 (59–115)	73 (26–114)
Total sperm count (million/ ejaculate)	261 (144–402)	185 (75–336)
Progressive sperm motility (%)	45 (38–51)	29 (19–37)
Normal forms (%)	21 (18–23)	15 (12–18)

[a] NSP: men with sperm concentration ≥ 15 mil/mL, Total sperm count ≥ 39 mil/ejaculate, Progressively sperm motility ≥ 32% and Normal forms ≥ 4%.
[b] ASP: A man may contribute data to more than one category.
[c] Median and interquartile range (IQR, 25th-75th percentile).

levels had the same magnitude with the exception in patients with asthenozoospermia (Progressive motility < 32%) who showed higher Pb median levels in the seminal plasma (21.15 μg/L vs. 2.91 μg/L). The levels of Cd in the various matrixes showed that it had essentially the same magnitude in men with Total sperm count < 39 mil/ejaculate or Progressive sperm motility < 32%. In particular, these men had higher seminal plasma Cd levels compared to men with Total sperm count > 39 mil/ejaculate and progressive sperm motility > 32% (1.43 vs. 0.66 μg/L and 1.40 vs. 0.43 μg/L, respectively). Blood and seminal plasma Se levels were lower in men with oligozoospermia or asthenozoospermia compared to normozoospermic men. The levels of Hg in the various matrixes had essentially the same magnitude with the exception of the blood levels that were slightly higher than normozoospermic men. As levels were higher in both blood (6.74 vs. 4.46 μg/L) and seminal plasma (2.36 vs. 1.86 μg/L) of men with asthenozoospermia compared to men with progressive sperm motility > 32%. Blood Ni levels were higher in men with abnormal sperm concentration (12.30 vs. 7.69 μg/L), total sperm count (19.07 vs. 7.04 μg/L) or sperm progressive motility (14.50 vs. 2.75 μg/L), compared with men with normal ones. In contrast, Ni levels had essentially the same magnitude in the seminal plasma of men with normal sperm parameters or oligozoospermia/asthenozoospermia. Sperm Ni level (0.088 vs. 0.040 μg/L) was higher in men with total sperm count < 39 mil/ejaculate than in reference category (total sperm count > 39 mil/ejaculate). Blood V levels were similar in NPS and in men with oligozoospermia. Men with asthenozoospermia had higher blood V levels than men with normal progressive sperm motility (9.76 vs. 5.85 μg/L). Seminal plasma V levels were higher in men with oligozoospermia (7.63 μg/L vs. 4.16 μg/L) than in men with Sperm concentration > 15 mil/mL. Finally, sperm V level were higher in men with sperm concentration < 15 mil/mL than in men with normal sperm concentration (0.011 vs. 0.007 μg/L).

3.3. Correlation between metal/metalloid levels and risk of abnormal sperm parameters associated with seminal plasma metals/metalloids exposure

Correlation between metal/metalloid levels in blood and seminal plasma is showed in Table S3.

We found several positive and negative correlations between the levels of metals/metalloids both in the same biological liquid and among the biological liquids investigated, the strongest ones are mentioned below (Table S3).

In particular, we found a moderately strong positive correlations between Pb seminal plasma levels with Cd seminal plasma levels (Rho: 0.773, p = 0.00) and with V blood levels (Rho: 0.645, p = 0.00). Conversely, it was found a moderately strong negative correlation between Pb and Se seminal plasma levels (Rho: − 0.698, p = 0.00).

Table 5 shows the risk of abnormal sperm parameters associated with seminal plasma metals/metalloids exposure. Vanadium had a positive unclear association with abnormal sperm concentration (OR = 2.28, 95% CI 0.69–7.59) and normal forms (OR = 2.16, 95% CI 0.76–6.10) only for the category 50th-70th. Cd showed increased risk of low total sperm count (OR = 4.48, 95%CI 0.25–80) and progressive sperm motility (OR = 3.45, 95%CI 0.77–16). Finally, Pb was associated with increased risk of low progressive sperm motility (OR = 4.51, 95%CI 0.86–23). Overall, these associations remained suggestive after adjustment, though statistically unstable risks (Table 5). The analyses of all elements investigated and the risk of abnormal sperm parameters is reported in Table S4.

Finally, we found weak interactions between beneficial effects of Se and detrimental ones only for Cd and Pb blood level on sperm concentration (Rho Cd: 0.03 p = 0.70 vs Rho Cd*Se: 0.29 p = 0.04; Rho Pb: − 0.05 p = 0.57 vs Rho Pb*Se: 0.122 p = 0.38), total sperm count (Rho Cd: − 0.06 p = 0.44 vs Rho Cd*Se: 0.16 p = 0.26; Rho Pb: − 0.01 p = 0.91 vs Rho Pb*Se: 0.11 p = 0.42), and progressive sperm motility (Rho Cd: 0.08 p = 0.33 vs Rho Cd*Se: 0.93 p = 0.00; Rho Pb: 0.02 p = 0.77 vs Rho Pb*Se: 0.19 p = 0.17).

4. Discussion

In this study we found that the exposure to Pb and Cd, both to environmental and occupational reasons, may contribute to a decline in human semen quality, whereas Se may have beneficial effects.

These results suggest that environmental pollutants may have a negative influence on sperm parameters, in line with literature (Mendiola et al., 2011; Deng et al., 2016; Wang et al., 2017; Zhang et al., 2020). In particular, in 2011 Mendiola and colleagues reported a significant positive correlation between seminal plasma levels of Pb and Cd and the percentage of immotile spermatozoa (Mendiola et al., 2011). In a 2016 systematic review and meta-analysis, due to the limited literature available, the authors did not found significant differences in sperm parameters between the group of men exposed to air pollution and the control group of non-exposed, although there was a trend towards a worsening of sperm concentration, total and progressive motility and normal morphology (Deng et al., 2016). Similar results were reported in 2017 by Wang and colleagues, who found an inverse linear dose-dependent relationship between seminal plasma As and Cd levels and progressive and total sperm motility (Wang et al., 2017). A recent meta-analysis and systematic review also reported an association between air pollution and reduction of sperm volume, concentration,

Table 4
Distribution of metal/metalloid levels in blood (B), seminal plasma (SP) and spermatozoa (S) (μg/L and μg/g for spermatozoa), by sperm parameters.

Metals/ metalloids	Sperm concentration		Total sperm count		Progressive sperm motility		Normal forms[a]	
	(< 15 mil/mL) n = 21	(> 15 mil/mL) n = 152	(< 39 mil/ ejaculate) n = 14	(> 39 mil/ ejaculate) n = 159	(< 32%) n = 83	(> 32%) n = 96	(< 16%[a]) n = 90	(> 16%[a]) n = 81
As (B)	3.84 (2.39–6.52)	6.00 (3.24–9.59)	5.12 (2.96–9.49)	5.58 (2.88–9.22)	6.74 (3.87–9.74)	4.46 (2.44–8.62)	5.48 (2.65–9.55)	5.94 (3.55–8.52)
As (SP)	0.41 (0.40–1.77)	2.46 (0.40–5.11)	0.76 (0.40–1.77)	2.34 (0.40–4.98)	2.36 (0.59–5.71)	1.86 (0.40–3.90)	1.44 (0.40–5.23)	2.75 (0.67–4.73)
As (S)	0.008 (0.003–0.019)	0.007 (0.003–0.019)	0.007 (0.003–0.036)	0.007 (0.003–0.019)	0.007 (0.002–0.020)	0.007 (0.003–0.019)	0.007 (0.002–0.015)	0.008 (0.003–0.022)
Cd (B)	0.32 (0.19–0.90)	0.32 (0.16–0.77)	0.33 (0.32–0.77)	0.32 (0.16–0.78)	0.280 (0.180–0.720)	0.370 (0.175–0.900)	0.33 (0.18–0.76)	0.28 (0.16–0.86)
Cd (SP)	0.49 (0.19–2.02)	0.75 (0.32–1.65)	1.43 (0.096–3.65)	0.66 (0.32–1.61)	1.40 (0.45–2.59)	0.43 (0.27–0.90)	0.68 (0.38–1.53)	0.70 (0.28–2.05)
Cd (S)	0.009 (0.006–0.028)	0.006 (0.004–0.015)	0.013 (0.006–0.033)	0.006 (0.004–0.014)	0.006 (0.003–0.020)	0.007 (0.005–0.014)	0.006 (0.004–0.013)	0.007 (0.004–0.017)
Hg (B)	5.73 (2.99–10.37)	3.42 (1.94–9.15)	5.70 (2.97–10.41)	3.2 (1.26–7.01)	4.60 (2.12–10.94)	5.77 (3.10–9.09)	4.96 (2.24–8.38)	6.08 (3.12–13.10)
Hg (SP)	1.20 (1.20–9.39)	1.20 (1.20–3.13)	1.20 (1.20–9.18)	1.20 (1.20–3.21)	1.20 (1.20–2.41)	1.20 (1.20–4.59)	1.20 (1.20–3.50)	1.20 (1.20–2.58)
Hg (S)	0.017 (0.013–0.030)	0.014 (0.009–0.023)	0.021 (0.015–0.037)	0.014 (0.009–0.021)	0.014 (0.010–0.030)	0.014 (0.009–0.022)	0.013 (0.008–0.019)	0.016 (0.010–0.040)
Ni (B)	12.30 (2.75–24.89)	7.69 (2.75–18.51)	19.07 (2.75–25.29)	7.04 (2.75–18.45)	14.50 (2.75–22.94)	2.75 (2.75–12.56)	7.35 (2.75–21.88)	8.00 (2.75–18.00)
Ni (SP)	2.68 (2.68–7.91)	2.68 (2.68–8.55)	2.68 (2.68–9.46)	2.68 (2.68–8.35)	2.68 (2.68–8.59)	2.68 (2.68–8.20)	2.68 (2.68–8.13)	2.68 (2.68–8.95)
Ni (S)	0.050 (0.032–0.113)	0.040 (0.026–0.094)	0.088 (0.042–0.159)	0.040 (0.027–0.093)	0.038 (0.023–0.108)	0.042 (0.031–0.095)	0.039 (0.025–0.084)	0.045 (0.029–0.127)
Pb (B)	13.7 (6.1–23)	14.14 (7.0–27)	13.74 (8.29–18.42)	11.08 (6.88–18.27)	10.70 (6.18–17.68)	12.62 (7.29–18.71)	11.02 (7.12–17.57)	12.28 (6.52–19.46)
Pb (SP)	13.18 (0.45–25.99)	13.73 (0.45–27.52)	14.23 (0.45–119)	13.61 (0.45–25.47)	21.15 (1.62–42.33)	2.91 (0.45–13.97)	13.55 (0.45–22.85)	13.77 (0.45–30.13)
Pb (S)	0.01 (0.01–0.03)	0.01 (0.01–0.04)	0.029 (0.007–0.110)	0.014 (0.006–0.038)	0.017 (0.006–0.060)	0.012 (0.007–0.039)	0.015 (0.006–0.029)	0.014 (0.006–0.053)
Se (B)	73.43 (62.63–142)	117 (82.21–143)	77.7 (65.13–121.34)	117.19 (78.54–144.60)	110 (76.51–144)	115 (76.66–142)	119.62 (80.85–146.98)	89.44 (72.49–130.83)
Se (SP)	1.18 (1.18–13.04)	4.83 (1.18–20.91)	1.18 (1.18–9.39)	4.94 (1.18–19.98)	1.18 (1.18–12.71)	9.10 (1.18–23.46)	1.18 (1.18–13.98)	6.80 (1.18–35.66)
Se (S)	0.114 (0.032–0.263)	0.211 (0.131–0.368)	0.128 (0.0332–0.278)	0.204 (0.121–0.361)	0.210 (0.128–0.389)	0.172 (0.109–0.352)	0.151 (0.097–0.338)	0.232 (0.145–0.389)
V (B)	5.66 (0.39–9.68)	8.00 (2.52–12.17)	5.32 (0.39–10.16)	7.72 (2.33–12.08)	9.76 (5.76–13.14)	5.85 (0.39–9.62)	6.23 (0.39–12.06)	8.50 (5.50–11.94)
V (SP)	7.63 (3.09–17.45)	4.16 (2.11–15.72)	3.91 (0.92–12.41)	4.49 (2.24–15.89)	3.60 (1.61–9.19)	5.43 (3.45–17.86)	6.03 (2.95–17.03)	3.55 (1.85–12.23)
V (S)	0.011 (0.007–0.033)	0.007 (0.005–0021)	0.014 (0.007–0.039)	0.007 (0.005–0.021)	0.007 (0.004–0.028)	0.008 (0.006–0.020)	0.007 (0.005–0.022)	0.008 (0.005–0.021)

The metal/metalloid levels are reported as median and interquartile range (IQR, 25th–75th percentile). Number of participants included in each analysis do not match to the total sample size because of missing data.

[a] For morphology/normal forms, we used the median value 16% because none of the men enrolled in this study had a percentage of normal forms lower than reference value (4%)

motility and normal morphology (Zhang et al., 2020).

In the last decades, a growing interest developed on the effects that environmental pollutants have on human health and in particular on male fertility, also due to the emergence of evidence in favor of a significant decline (50–60%) of male fertility in Western countries over the last 40 years (Levine et al., 2017). However, the evidences about the relationship between environmental pollution and male fertility are often conflicting due to variations in sample size studied, composition of study population and to the assessed exposure markers.

The exact mechanism by which environmental pollutants exert their harmful effects on the reproductive system is not entirely clear. It may be ascribed to an alteration of the endocrine axis that regulate spermatogenesis and/or to a direct testicular toxicity with inhibition of androgen biosynthesis in Leydig cells. It is also known that metals/metalloids may increase the oxidative stress and the lipid peroxidation (Pant et al., 2015; Lanzafame et al., 2009).

Pb is a ubiquitous heavy metal highly harmful for the human health. According to the World Health Organization (WHO), Pb exposure accounted for 1.06 million deaths and 24.4 million years of healthy life lost (WHO, 2017). In addition to occupational exposure, Pb comes from foods, particularly seafood from metal-polluted areas, water, air of areas with high gasoline vehicle traffic, coatings often used for housing, and tobacco smoke (WHO, 2017). To date there is no single safe level of Pb exposure; e.g. in 2015, NIOSH designated 5 μg/dL of whole blood, as the reference blood lead level for adults, whereas data from the National Health and Nutrition Examination Survey (NHANES) show that the average blood lead levels (geometric mean) of all adults in the United States in 2009–2010 was 1.2 μg/dL" [https://www.cdc.gov/niosh/topics/ABLES/description.html]. According to the recent literature, Pb levels $\geq$ 10 μg/dl damage male reproduction and other evidences reported adverse effect (decreased acrosome reaction and sperm fertilization rates) also for Pb levels $\geq$ 5 μg/dl (Kumar, 2018; Godínez-Solís et al., 2019). Indeed, even at relatively low blood levels, Pb can exert its harmful effects on human fertility, particularly it worsens conventional sperm parameters, increases oxidative stress-related sperm DNA fragmentation, unpacks chromatin, induces an abnormal acrosome reaction, decreases prostate secretion, and it lowers libido (Awadalla et al., 2011; Kumar, 2018). Moreover, in female partners of chronically exposed workers, Pb seems to increase miscarriages rate and to decrease fertility rate (Awadalla et al., 2011).

Table 5
Odds ratio (ORs) and 95% confidence interval (CI) for abnormal sperm parameters and seminal plasma metals/metalloids levels (μg/L).

	N[a] </>	OR_{crude} (95% CI)	p-value	OR_{adj} (95% CI)	p-value
Sperm concentration[b,c]					
V					
< 50th	11/98	1		1	
50th–75th	2/16	2.29 (0.75–7.05)	0.15	2.29 (0.69–7.59)	0.18
> 75 th	8/38	1.95 (0.64–5.95)	0.24	0.73 (0.13–3.94)	0.71
Total count[b,d]					
Cd					
< 50th	5/78	1		1	
50th–75th	1/42	0.37 (0.04–3.28)	0.37	2.15 (0.41–11)	0.37
> 75th	6/35	2.67 (0.77–9.35)	0.12	4.48 (0.25–80)	0.31
Progressive motility[b,e]					
Pb					
< 50th	25/61	1		1	
50th–75th	22/20	2.68 (1.25–5.76)	0.01	3.01 (0.97–9.37)	0.06
> 75th	36/15	5.86 (2.74–12)	0.00	4.51 (0.86–23)	0.08
Cd					
< 50th	25/60	1		1	
50th–75th	23/20	2.76 (1.29–5.90)	0.01	2.45 (1.02–5.92)	0.05
> 75th	31/10	7.44 (3.17–17)	0.00	3.45 (0.77–16)	0.11
Normal forms[b,f]					
V					
< 50th	35/48	1		1	
50th–75th	25/16	2.14 (0.99–4.60)	0.05	2.16 (0.76–6.10)	0.15
> 75th	30/17	2.42 (1.16–5.06)	0.02	0.54 (0.12–2.46)	0.43

[a] Below/above sperm parameter reference in normal men. Number of participants included in each analysis do not match to the total sample size because of missing data.
[b] Crude model, 50th as reference exposure category.
[c] Adjusted for cryptorchidism, obesity, alcohol consumption, smoke and Se.
[d] Adjusted for Se, alcohol consumption, obesity and cryptorchidism.
[e] Adjusted for industrial workers/professional exposure, hashish, Pb, Cd and Se.
[f] Adjusted for industrial workers, alcohol consumption, varicocele, BMI, hashish, endocrine disorders, cryptorchidism and Se.

Based on these data, Awadalla and colleagues conducted a cross-sectional study in 29 infertile male patients, who compiled a questionnaire about age, residence area (rural or urban), smoking habits and type of occupation farmers, blue collar workers, specifically mechanics, painters and carpenters, or white collar workers. The enrolled patients were divided into two groups depending on whether Pb blood levels were higher or lower than 20 mcg/dl. Each patient underwent to conventional sperm parameters evaluation and sperm DNA integrity assessment by flow cytometry. The study showed that sperm concentration, motility and morphology were not statistically different in the two groups, whereas a significantly greater percentage of spermatozoa with DNA fragmentation was found in the patients with high Pb blood levels (Awadalla et al., 2011). Blood levels of Pb correlated with those in semen, as already previously reported (Benoff et al., 2000). Differently to what reported by Awadalla (Awadalla et al., 2011), Telisman and colleagues, after evaluating the semen parameters of 143 healthy male industrial workers (98 with slight-to-moderate occupational exposure to Pb and 51 controls), showed that low-to-moderate levels of Pb exposure (< 400 mcg/L) significantly altered all conventional sperm parameters (Telisman et al., 2007). Finally, some studies reported a reproductive damage secondary to a Pb-induced hormonal imbalance (Kumar, 2018).

Pb could act by altering the synthesis of DNA in sperm precursors or interfering with the chromatin condensation. Pb competes with zinc and binds protamine (which protects DNA), causing a conformational change of the protein resulting in an abnormal chromatin condensation (Awadalla et al., 2011). Furthermore, Pb and, more generally, many heavy metals, increase reactive oxygen species production (ROS) and decrease antioxidant mechanisms in the seminal plasma (Calogero et al., 2011). Experimental studies suggest that some Pb-induced toxic effects could be, at least partially, counteracted by the administration of antioxidants, such as vitamin C plus thiamine as well as thiamine or vitamin E plus zinc by inhibiting cell apoptosis (Dhawan et al., 1988; Shan et al., 2009). In our study, we found that Pb levels were higher in the seminal plasma of patients with asthenozoospermia and it was associated with low sperm motility.

Cd is ubiquitous, although the main source is tobacco smoke. It is associated with changes in sperm parameters and reproductive toxicity, also because of the very long biological half-life (from 10 to 40 years) and the consequent bioaccumulation (De Angelis et al., 2017). The correlation between Cd and sperm parameters is still unclear. Previous studies have shown significant negative effects of low to moderate Pb and Cd exposure on semen quality in Indian, Mexican, Croatian, American and Spanish populations (Telisman et al., 2007; Jurasović et al., 2004; Pant et al., 2003; Hernández-Ochoa, 2005; Benoff et al., 2009; Mendiola et al., 2011). Similarly, several studies have shown an association between impaired sperm motility and Cd and/or Pb levels in seminal fluid (Hernández-Ochoa et al., 2005; Pant et al., 2003; Benoff et al., 2009; Wang et al., 2016). The levels of Pb and Cd in seminal plasma, evaluated in 46 infertile patients and 73 fertile men, were significantly higher in the infertile patients and a significant inverse correlation was found between the levels of these metals and sperm concentration and motility (Pant et al., 2015). Xu and colleagues had also found that seminal plasma Cd could affect sperm quality and cause oxidative stress-induced DNA damage in human spermatozoa (Xu et al., 2003). On the contrary, in the past years, a relationship between conventional sperm parameters and seminal plasma levels was not found (Keck et al., 1995; Hovatta et al., 1998). Studies on animal models showed that Cd accumulated in the testes and epididymis, inducing degeneration of the seminiferous tubules, destruction of testicular cell junctions, including Sertoli cell-blood-testis barrier (Manfo et al., 2014) and steroidogenesis impairment, resulting in lower testosterone levels (Gunnarsson et al., 2004; Manfo et al., 2014). Regarding the possible mechanisms of Cd toxicity, animal studies suggest that Cd, similarly to Pb, causes mitochondrial dysfunction, increases ROS and reduces antioxidant defenses, causes lipid peroxidation of the cell membrane and oxidative DNA damage (Pant et al., 2015). Cd also acts as endocrine disruptors, since it impaired steroidogenesis and hypothalamus – pituitary – gonadal axis function (De Angelis et al., 2017; Kumar and Sharma, 2019). We found that the levels of Cd in blood and spermatozoa had essentially the same magnitude in ASP and NSP groups. We also found that men with asthenozoospermia had higher seminal plasma Cd levels than men who had these parameters in the normal range.

Harmfull effects of Cd and Pb seem to be partly counteracted by Se, an essential element for male fertility and for spermatogenesis (Mirnamniha et al., 2019). Se, in the form of selenocysteine, acts as the catalytic center in the active sites of glutathione peroxidase antioxidant enzymes (Cohen and Takahashi, 1986; Zhang et al., 1989; Chu et al., 1996; Mirnamniha et al., 2019). A positive correlation between seminal Se levels, and sperm concentration, total sperm number, and motility was reported. Hence, Se could protect against oxidative DNA damage in human spermatozoa (Xu et al., 2003). Eroglu and colleagues found that blood and semen levels of Se correlate positively with spermatozoa concentration, motility and morphology (Eroglu et al., 2014). Recently,

Morbat and colleagues showed that Se supplementation was associated with significant improvement of sperm parameters (Morbat et al., 2018). According to these evidences, we found that blood and seminal plasma Se levels were lower in men with abnormal sperm parameters.

Arsenic (As) is also on the list of endocrine disruptors. It alters steroid and thyroid hormone receptor-mediated gene regulation (Jeng et al., 2015) and it seems to be associated with a worsening of conventional sperm parameters, in particular sperm concentration (Xu et al., 2012) and a dose-dependent reduction of sperm motility (Renu et al., 2018). Experimental studies in animal suggest that also for As the mechanism of toxicity is linked to the oxidative damage and that treatment with ascorbic acid is beneficial, as it can reduce the oxidative stress (Chang et al., 2007). A recent review showed that As could inhibit spermatogenesis process, reduce testosterone synthesis, impair sperm capacitation and it generates oxidative stress by reducing antioxidant enzymes levels (Renu et al., 2018). In our study, we found higher blood As levels in men with abnormal sperm parameters than in normozoospermic men.

The main sources of Hg are seafood, alcohol, coffee, and dental amalgam fillings (Henriques et al., 2019). In-vitro and animal model studies have shown a negative impact of this element on the male reproductive system (Henriques et al., 2019). Particularly, it induces DNA breaks in spermatozoa and leads to decreased sperm motility and viability (Mocevic et al., 2013). Human studies are still too few and have shown conflicting results. A case-control study showed high levels of Hg in the seminal plasma of infertile patients, associated with sperm parameter abnormalities (Choy et al., 2002). The mechanism of toxicity on the male reproductive system is represented by disruptions of sperm membrane permeability and alteration of mitochondrial function and DNA synthesis (Choy et al., 2002). However, other studies did not report toxic effects of Hg on male fertility and no study showed correlation between Hg exposure and hormonal alterations (Mocevic et al., 2013).

In the present study, we found that blood Hg levels were slightly higher in men with abnormal sperm parameters. This finding is similar to that of Mendiola and colleagues, who did not report an association between blood or seminal plasma Hg levels and sperm quality (Mendiola et al., 2011).

Vanadium (V) is among the main environmental pollutants. Its main sources of contamination for people are atmospheric dust, food and smoking (Chandra et al., 2007a). In-vivo studies exploring the toxic effects of V on the reproductive system are still few, but they seem to link to the gametes formation. A study conducted on rats showed that V is responsible for testicular damage, characterized by the presence of cell degeneration, destruction of the germinal epithelium in the seminiferous tubules with moderate-to-severe tubular necrosis and Leydig cells hyperplasia (Chandra et al., 2007a). The authors also found a decreased sperm concentration, a marked increase in lipid peroxidation and decreased superoxide dismutase and catalase activity. Furthermore, the administration of zinc seems to play a protective effect (Chandra et al., 2007b). More recently, Wang and colleagues found that high seminal plasma V levels were associated with risk of worse sperm parameters, sperm DNA damage and hormonal imbalance (Wang et al., 2018). According to these results, we found higher V levels in seminal plasma and spermatozoa of men with abnormal sperm parameters. Moreover, blood V levels were higher in men with asthenozoospermia than in men with normal sperm motility.

Finally, there are still few studies exploring the effects of Ni and most of them have been conducted on animals. It seems that exposure to this metal, especially for employment reasons (welders), is associated to sperm morphological abnormalities (Danadevi et al., 2003; Slivkova et al., 2009) and to a decreased sperm progressive motility (Danadevi et al., 2003). Sperm abnormalities correlate with the number of years of exposure to welding fumes containing nickel and chromium (Danadevi et al., 2003). In contrast, Bian et al. (2019) reported for the first time that Ni could improve sperm motility. We found that blood and sperm Ni levels were higher in men with abnormal sperm parameters than in normozoospermic men, whereas seminal plasma levels were similar between the two groups.

Several limitations should be mentioned. The cross-sectional design of the study does not allow establishing any temporal relationships between exposure and outcome, which restricts the feasibility of establishing causal relationships. We made a single specimen collection per participant; therefore the measurements of elements may fail to account for variable time exposures and elimination half-lives of metals/metalloids, often leading to exposure misclassification and attenuation of risk estimates. Unfortunately, we had no measurements of zinc, therefore we cannot take into account its impact on the harmful effects of metals/metalloids on sperm quality, leading to some exposure misclassification. Unfortunately our study had a recruitment bias relative to smokers, in fact our sample had 44.8% of smokers, and unexpectedly they were more numerous in the rural area than in the industrial one (Table S1). Moreover, both smokers and former were slightly more numerous in the NSP group than in the ASP one (Table 2), for this reason we estimated the ORs using the conventional sperm parameters, dichotomized as below at or above the WHO reference values, as an independent variable.

The major strength of our study was the simultaneous measurements of metals/metalloids in blood and seminal fluid, thus allowing us to explore the best estimate of the exposure status of the male reproductive system. We recruited participants from general population, which doesn't limit the generalization of the findings to the general population.

5. Conclusion

This study confirms the impact of metals/metalloids exposures at environmental levels on the conventional human semen-quality parameters. In particular, our findings suggest that environmental exposures to Pb, and Cd are associated with below-reference sperm quality parameters, whereas Se can have beneficial effects. Measurements of metals/metalloids in the seminal fluid may be more predictable of semen quality than conventional blood measurements. Finally, the association between blood, seminal plasma and sperm metal/metalloid levels and sperm quality sometimes is unclear, so further studies are needed.

CRediT authorship contribution statement

Aldo E. Calogero: Conceptualization, Methodology, Writing - review & editing, Project administration; **Maria Fiore:** Formal analysis, Writing - original draft preparation, Writing - review & editing; **Filippo Giacone:** Recruitment; **Maria Altomare**: Data curation; **Paola Asero:** Visualization; **Caterina Ledda:** Formal analysis; **Giulietta Romeo:** Visualization; **Laura M. Mongioì:** Visualization; **Chiara Copat:** Writing - review & editing; **Maria Giuffrida:** Visualization; **Enzo Vicari:** Visualization; **Salvatore Sciacca:** Conceptualization, Methodology, Writing - review & editing; **Margherita Ferrante**: Supervision.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112165.

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