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Biomonitoring of Urinary Organic and Inorganic Arsenic in Four Different Polluted Areas in Italy

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INTRODUCTION

Arsenic and its inorganic compounds are classified as carcinogenic to humans. Exposures to inorganic arsenic (iAs) in drinking water are associated with both carcinogenic and non-carcinogenic effects. The risk assessment of exposures to low-moderate levels of environmental arsenic (As) is a challenging objective for research and public health. Therefore an epidemiological study based on Human Biomonitoring Survey (**SEpiAs study***) was carried out in four areas of Italy (figure 1).
Source of As is natural in Amiata and Viterbese (soil and water), anthropogenic in Taranto and Gela. A contamination of tap water by arsenic is documented in Viterbese where mean value significantly exceed 10 µg/l (*law limit* established by the Directive 98/83/CE). Gela and Taranto were declared as Reclamation Sites of National Interest characterized by industrial areas (Refinery, steel plant) on the basis of documented soil contaminations or presence of hazardous waste (figures 2 and 3). Numerous epidemiological studies have reported health risks in the four areas.

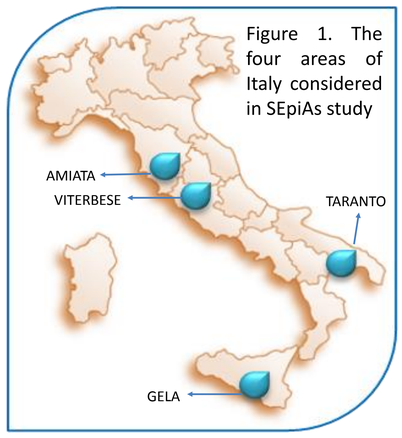


Figure 1. The four areas of Italy considered in SEpiAs study

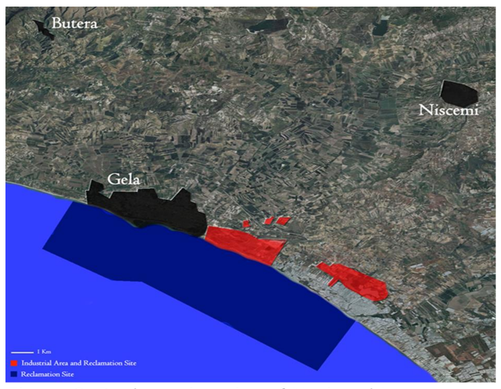


Figure 2. Reclamation site of National Interest of Gela, Niscemi, Butera

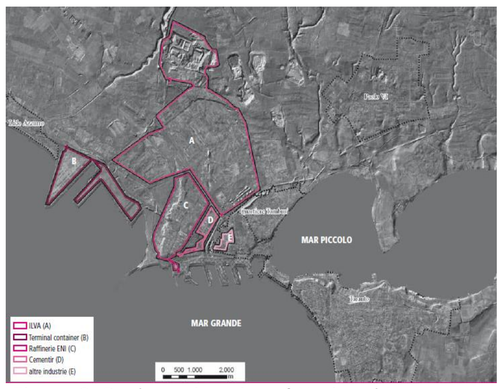


Figure 3. Reclamation site of National Interest of Taranto

OBJECTIVES

The main objectives are the:
- description of As distribution by area;
- identification of subjects with high level of iAs;
- evaluation of the relationship between arsenic concentration and factors of exposure;
- development of indicators for environment and health monitoring system.

METHODS

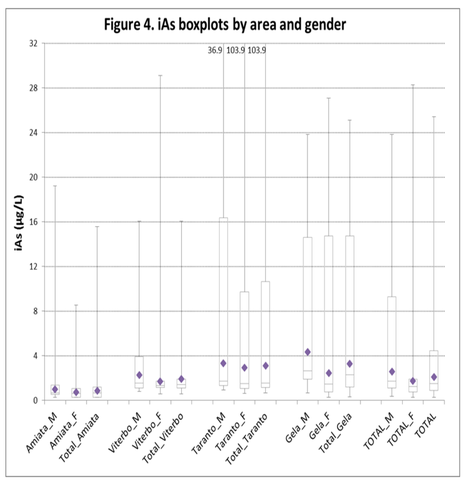
The study design is a multicentric observational epidemiological survey based on the measure of biological markers. 271 subjects (132 men) aged 20-44, were randomly sampled stratifying by area, gender and age classes (20-29, 30-39, 40-44 years) (table 1). Individual data on residential history, socio-economic status, environmental and occupational exposures, lifestyle and dietary habits, were collected through interviews using questionnaire. In urine samples of recruited subjects, the concentration of iAs and methylated species (MMA, DMA) was measured using inductively coupled mass spectrometer (DRCICP-MS), after chromatographic separation (HPLC).
Genetic susceptibility was evaluated by a set of polymorphism reported in scientific literature as associated with As (AS3MTMet287Thr, GST-T1, GST-M1, OGG1).
The distributions of iAs and iAs+MMA+DMA were described by area and gender using Geometric Mean (GM) and percentiles (50p, 75p, 95p).
The associations between As species and exposure variables were evaluated by GM Ratio (GMR), adjusted for genetic susceptibility and fish consumption in the three days before the urine sample taken.

Table 1. Sample units by area, gender and age classes

Area	Gender and age classes											
	M				F				Total			
	20-29	30-39	40-44	Total	20-29	30-39	40-44	Total	20-29	30-39	40-44	Total
Amiata	10	12	6	**28**	11	11	8	**30**	21	23	14	**58**
Viterbese	15	11	6	**32**	16	15	9	**40**	31	26	15	**72**
Taranto	11	9	4	**24**	11	10	5	**26**	22	19	9	**50**
Gela	14	20	14	**48**	24	11	8	**43**	38	31	22	**91**
Total	**50**	**52**	**30**	**132**	**62**	**47**	**30**	**139**	**112**	**99**	**60**	**271**

RESULTS

Figure 4. iAs boxplots by area and gender



Taranto and Gela samples showed the highest variability of iAs.

High heterogeneity among areas was observed.(Figure 4).

Gela and Taranto samples had higher iAs concentration (Gela: GM=3.3 µg/L, 75p=14.7 µg/L; Taranto: GM=3.1 µg/L, 75p=10.7 µg/L) than Viterbo (GM=1.9 µg/L, 75p=1.9 µg/L) and Amiata (GM=0.9 µg/L, 75p=1.2). Males of Taranto and Gela had the highest GM of iAs (Taranto: GM= 3.32 µg/L, 75p=19.0; Gela: GM=4.3 µg/L, 75p=14.6 µg/L) .

Similar results were observed using iAs+MMA+DMA .

Table 2. Number and percentage of sample units with iAs>1.5 µg/L by gender and area.

	Amiata n(%)	Viterbese n(%)	Taranto n(%)	Gela n(%)	Total n(%)
Male	7(25.0)	18(56.3)	15(62.5)	41 (85.4)	**81(61.4)**
Female	5(16.7)	17(42.5)	14(53.8)	20(46.5)	**56(40.3)**
Total	**12(20.7)**	**35(48.6)**	**29(58.0)**	**61(67.0)**	**137(50.6)**

Table 3. Number and percentage of sample units with iAs+MMA+DMA>15 µg/L by gender and area.

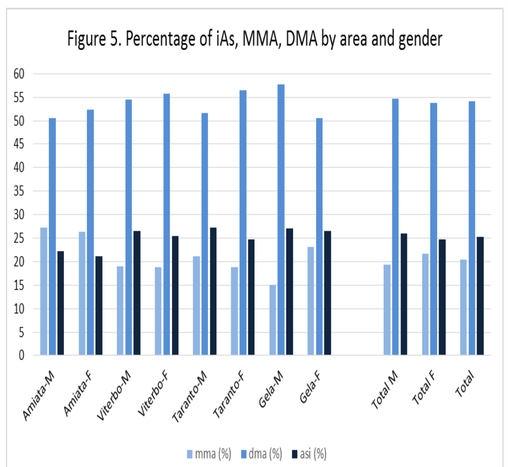
	Amiata n(%)	Viterbese n(%)	Taranto n(%)	Gela n(%)	Total n(%)
Male	4(14.3)	7(21.9)	8(33.3)	21(43.8)	**40(30.3)**
Female	3(10.0)	3(7.5)	7(26.9)	15(34.9)	**28(20.1)**
Total	**7(12.1)**	**10(13.9)**	**15(30.0)**	**36(39.6)**	**68(25.1)**

Table 4. Number and percentage of sample units with iAs>3.86 µg/L by gender and area.

	Amiata n(%)	Viterbese n(%)	Taranto n(%)	Gela n(%)	Total n(%)
Male	4(14.3)	8(25.0)	8(33.3)	21(43.8)	**41(31.1)**
Female	3(10.0)	3(7.5)	7(26.9)	15(34.9)	**28(20.1)**
Total	**7(12.1)**	**11(15.3)**	**15(30.0)**	**36(39.6)**	**69(25.5)**

Subjects with iAs > 1.5 µg/L (reference value reported by Italian Society of Reference Value - ISRF) and iAs+MMA+DMA>15 µg/L (ISRF) were 137 (50.6% of total sample) and 68, 25.1% of total sample (tables 2 and 3). Subjects with iAs>3.86 µg/L (reference value identifying subjects with high priority of survaillance – Hays et al. 2010) were 69 (25.5%) (table 4).

Figure 5. Percentage of iAs, MMA, DMA by area and gender



The percentages of iAs and MMA on total As were respectively 25.3% and 20.5%, close to the upper limit of ranges reported in scientific literature (respectively 10%-30%, 10-20%).
In males of Taranto and Gela the percentages of iAs were the highest (about 27%, figure 5).

*"Studies on markers of exposure and early effect in areas with arsenic pollution: methods and results of the project SEpiAs". *Epidemiol Prev* 2014; 38 (3-4).

Table 5. Associations between iAs and exposure variables using adjusted GMR, 95%CI - All Areas

Questionaire Variables	Gender	exposure	n	%	MG	GMR	p	95%CI
Exposure to Inorganic solvents, acids	M+F	NO	216	80.9	1.86			
		YES	51	19.1	3.87	**1.55**	**0.037**	**1.03-2.33**
	M	NO	97	74.6	2.25			
		YES	33	25.4	4.20	1.31	0.308	0.78-2.21
	F	NO	119	86.9	1.59			
		YES	18	13.1	3.32	**2.02**	**0.042**	**1.03-3.98**
Exposure to petrol derivates	M	NO	97	74.6	2.11			
		YES	33	25.4	5.05	1.50	0.153	0.86-2.61
Occupational exposure in chemical industrials	M	NO	109	83.8	2.17			
		YES	21	16.2	7.25	**2.33**	**0.008**	**1.25-4.35**
Consumption of whole milk	M+F	NO	202	75.7	2.01			
		YES	65	24.3	2.57	1.54	**0.018**	**1.08-2.21**
	F	NO	108	78.8	1.50			
		YES	29	21.2	3.10	**2.53**	**<0.001**	**1.51-4.23**
Consumption of fish	M+F	NO	62	23.2	1.60			
		YES	205	76.8	2.33	**1.51**	**0.027**	**1.05-2.17**
	M	NO	27	20.8	1.62			
		YES	103	79.2	3.00	**2.05**	**0.007**	**1.22-3.43**
Consumption of fish of own/local production	M+F	NO	212	79.4	1.95			
		YES	55	20.6	3.07	1.56	0.105	0.91-2.67
	M	NO	100	76.9	2.37			
		YES	30	23.1	3.73	**2.47**	**0.046**	**1.01-6**
Consumption of fresh vetetable of own/local production	F	NO	100	73.0	1.49			
		YES	37	27.0	2.71	**1.86**	**0.026**	**1.08-3.22**
Consumption of fruit of own/local production	M	NO	94	72.3	2.38			
		YES	36	27.7	3.44	**2.04**	**0.027**	**1.09-3.83**
Consumption of bread/pasta of own/local production	M	NO	118	90.8	2.49			
		YES	12	9.2	4.63	**2.91**	**0.006**	**1.36-6.24**

Table 8. Associations between iAs and exposure variables using adjusted GMR, 95%CI - Taranto area

Questionaire Variables	Gender	exposure	n	%	MG	GMR	p	95%CI
Exposure to industrial dust, chemical substances, gas or radiations	M+F	NO	28	56.0	2.26			
		YES	22	44.0	4.73	2.12	0.103	0.85-5.29
	M	NO	11	45.8	2.16			
		YES	13	54.2	4.78	2.26	0.229	0.57-8.94
	F	NO	17	65.4	2.33			
		YES	9	34.6	4.67	2.72	0.153	0.67-11.05
Exposure to silica	M	NO	16	66.7	2.32			
		YES	8	33.3	6.83	***3.57***	***0.085***	***0.83-15.43***
Exposure to asbestos	M	NO	18	75.0	2.37			
		YES	6	25.0	9.20	***4.69***	***0.053***	***0.98-22.4***
Exposure to Inorganic solvents, acids	M+F	NO	36	72.0	2.62			
		YES	14	28.0	4.96	2.31	0.107	0.83-6.45
	M	NO	16	66.7	2.82			
		YES	8	33.3	4.62	1.68	0.491	0.36-7.83
	F	NO	20	76.9	2.46			
		YES	6	23.1	5.45	***4.23***	***0.092***	***0.78-23.1***
Consumption of whole milk	M+F	NO	40	80.0	2.53			
		YES	10	20.0	7.35	**3.21**	**0.049**	**1.01-10.25**
Consumption of fish of own/local production	M+F	NO	10	20.0	1.35			
		YES	40	80.0	3.86	***3.02***	***0.071***	***0.9-10.06***
Consumption of fresh vetetable of own/local production	M+F	NO	12	24.0	1.70			
		YES	38	76.0	3.79	2.22	0.142	0.76-6.51

Table 6. Associations between iAs and exposure variables using adjusted GMR, 95%CI - Amiata area

Questionaire Variables	Gender	exposure	n	%	MG	GMR	p	95%CI
Consumption of whole milk and derivates of own/local production	M+F	NO	51	87.9	0.73			
		YES	7	12.1	2.96	***4.13***	***0.001***	***1.86-9.17***
	M	NO	24	85.7	0.83			
		YES	4	14.3	3.40	***4.40***	***0.026***	***1.21-16.05***
	F	NO	27	90.0	0.65			
		YES	3	10.0	2.46	***3.67***	***0.024***	***1.21-11.15***
Consumption of pork of own/local production	M+F	NO	47	81.0	0.80			
		YES	11	19.0	1.19	***1.85***	***0.094***	***0.9-3.81***
	M	NO	23	82.1	0.84			
		YES	5	17.9	2.39	***2.94***	***0.094***	***0.82-10.54***
Consumption of bread/pasta of own/local production	M	NO	23	82.1	0.87			
		YES	5	17.9	2.07	2.40	0.178	0.65-8.86

Table 7. Associations between iAs and exposure variables using adjusted GMR, 95%CI - Viterbese area

Questionaire Variables	Gender	exposure	n	%	MG	GMR	p	95%CI
Consumption of tap water	M+F	NO	68	94.4	1.83			
		YES	4	5.6	4.61	1.65	0.259	0.68-4.01
Consumption of mineral water	M+F	NO	6	8.3	6.72			
		YES	66	91.7	1.72	0.54	0.124	0.24-1.19
Use of tap water to cook	M+F	NO	34	47.2	1.38			
		YES	38	52.8	2.60	**1.50**	**0.050**	**1-2.26**
Exposure to Inorganic solvents, acids	M+F	NO	69	95.8	1.77			
		YES	3	4.2	13.68	**4.16**	**0.017**	**1.31-13.24**
Consumption of whole milk	F	NO	30	75.0	1.60			
		YES	10	25.0	2.01	**2.40**	**0.020**	**1.16-4.96**
Consumption of fresh vetetable of own/local production	F	NO	36	90.0	1.52			
		YES	4	10.0	4.39	***2.52***	***0.066***	***0.94-6.79***

Table 9. Associations between iAs and exposure variables using adjusted GMR, 95%CI - Gela area

Questionaire Variables	Gender	exposure	n	%	MG	GMR	p	95%CI
Exposure to petrol derivates	M	NO	23	50.0	3.53			
		YES	23	50.0	6.05	***1.73***	***0.082***	***0.93-3.24***
Occupational exposure in chemical industrials	M	NO	29	63.0	3.24			
		YES	17	37.0	8.48	**2.45**	**0.005**	**1.33-4.54**
Consumption of whole milk	F	NO	36	87.8	2.05			
		YES	5	12.2	8.61	***3.44***	***0.071***	***0.9-13.21***
Consumption of fish	M+F	NO	26	29.9	2.45			
	M+F	YES	61	70.1	3.94	***1.71***	***0.090***	***0.92-3.19***
	M	NO	13	28.3	2.35			
	M	YES	33	71.7	6.03	**2.44**	**0.010**	**1.26-4.73**
Consumption of fruit	M	NO	5	10.9	1.97			
	M	YES	41	89.1	5.13	2.28	0.125	0.79-6.57
Consumption of fruit of own/local production	M	NO	40	87.0	4.23			
	M	YES	6	13.0	8.37	1.90	0.193	0.71-5.05

Total sample showed a significant increase of iAs for occupational exposures and some types of food (table 5).
Amiata sample showed a cluster of subjects with high iAs consuming food and milk derivates of local/own production (table 6).
Viterbo sample showed significant increase of iAs in subjects drinking tap water or using tap water to cook. Increase of iAS was reported for subjects consuming milk and fresh vegetable (table 7).
Taranto sample showed significant increase of iAs for subjects exposed to occupational factors and to consumption of milk and fish or vegetables of local/own production (table 8).
Gela sample showed significant increase of iAs for subjects exposed to occupational factors and to consumption of milk, fish and fruit (table 9).
Similar results were observed using iAs+MMA+DMA .

CONCLUSIONS

The result showed mean concentrations of iAs higher in areas characterized by anthropogenic As pollution than those characterized by natural sources.
The main sources of As exposure appear to be occupational and related to some type of food (fruit and vegetables) and beverages (water and milk). The results showed different exposure pathway among areas. Results are relevant to identify and take care of subjects outliers for inorganic-organic arsenic and to aim primary prevention measure for decreasing the level of population exposure.

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