

# Frog Gonad as bio-indicator of Sarno River Health

Trocchia Samantha, Labar Sofiane, Abdel Gawad Fagr K., Rabbito Dea, Ciarcia Gaetano, Guerriero Giulia

**Abstract**— The Sarno River is affected by an extreme environmental degradation as a result of uncontrolled agricultural activities and the outflow of industrial waste. Changes in the abundance indexes and biodiversity of frogs can be used as a measuring tool to determine water quality of the Sarno River. The aim of this study is to demonstrate the potential in using frog tissue as environmental indicators and to monitor changes in water quality of local streams in the development area of Sarno River. Thus, many samples are collected from sediment, water and frogs for metals analysis. To identify any differentiations of bioaccumulation of metals we have been analyzed skin and gonad of the more representative frog specie along the Sarno river, the barcoded *Pelophylax bergeri*. Results show that the degree of pollution in the Sarno river varies by zone, as well as by metal to metal, with contents that often are very elevated in frogs and higher in water than sediment. The concentrations of heavy metals, especially Arsenic (As) Chromium (Cr) and Zinc (Zn) were found higher in testis than skin of the collected frogs. Therefore, our results sustain the important role of frog gonads as a good bio-indicator of pollution. Frogs, *Pelophylax bergeri*, and water of Sarno River are very contaminated by arsenic and need to be urgently remediated.

**Index Terms**— Sarno river, arsenic, gonad, frog, barcoding, water quality, biodiversity.

## 1 INTRODUCTION

In general all rivers in the world are extensively used as a source of irrigation water and the majority are considered polluted. So, inorganic pollution is ubiquitous in our environment [1,2] and result from diverse activities, such as industrial discharges, foundry wastes, wearing of metal parts and equipments, paints, vehicular traffic, mining and rock weathering. These are subsequently deposited on soil surfaces and may be leached through municipal drainages to nearby ponds, streams and rivers which are common amphibian habitats and hiding places [3]. The major concern with heavy metals lies in their acute toxicity and their ability to bioaccumulate in biological systems [4]. Biological monitoring, when applied to the same community over time, may detect some biological modifications, showing that the community, and then the physical environment, has been stressed. As a consequence biological monitoring may be considered a useful "warning signal". Sometimes the nature of the stress could be chemical, identifying and quantifying the pollutants involves chemical monitoring. Therefore, it is necessary to combine both biological and chemical monitoring, because one method cannot replace the other [5], [6], [7]. The advantages and limits of the chemical

and biological monitoring methods are discussed in other papers [8], [9]. Amphibians, living in the fringes of populated cities, may come under pressure from a number of anthropogenic factors including pollution from heavy metals. Frogs have a wide variety of diet and live in rugged terrains such as swamps, tree, thickets [10], as well as unkempt lawns and human habitations thus exposing them to polluting activities. A semi-permeable and highly vascularized skin allows cutaneous respiration in amphibians and therefore, may confer them a high propensity to accumulate environmental pollutants in their tissues directly from water and moist surroundings. It is now commonly speculated that amphibian species undergo a global decline and loss of variability in their populations, attributed to habitat destruction, introduction of invasive species, over exploitation, emerging diseases, pathogens, climate change and environmental contamination. Pollutants including heavy metals have been linked with the presence of free radicals which may induce oxidative stress in biological systems [3]. A variety of factors such as geology, chemical reactivity, mineralogy, hydrology, topography, vegetation, land use pattern and biological productivity regulate the pollutant load of a river and groundwater [11], [12]. Heavy metals entering surface waters from natural (erosion of drainage basin) or anthropogenic sources become part of the water suspension and sediment system by absorption, precipitation and ion exchange processes [12], [13]. Contaminants in river systems can be investigated by analyzing either the water, sediment, soil and/or fauna species like frogs. So, numerous studies involve the assessment of the ecosystem sensibility to the contamination by heavy metals with the use of frogs as specific bio-indicators of pollution [14], [15].

Since its foundation (X-VII century b.C.) Naples has been affected by natural hazards, such as floods and earthquakes [16]. The Sarno River, which is the principal source of irrigation water, is considered the most polluted water course in Campania, located in southwestern Italy. Agricultural activities within the Sarno Basin have changed the aquatic ecosystem of

- Trocchia Samantha, EClab. Department of Biology., University of Naples Federico II, Naples, Italy
- Labar Sofiane, department of biology, University of El-Tarf, REDD Lab Annaba, Algeria. Interdept. Research Center for Environment (I.R.C.Env.), University of Naples Federico II, Naples, Italy. E-mail: [labar\\_sf@yahoo.fr](mailto:labar_sf@yahoo.fr)
- Fagr K. Abdel Gawad, department of Water Pollution Research, Centre of Excellence for Advanced Science, National Research Center (NRC), Giza, Egypt. E-mail: [fagrabd1-gawad@nrc.sci.eg](mailto:fagrabd1-gawad@nrc.sci.eg)
- Rabbito Dea, EClab. Department of Biology, University of Naples Federico II, Naples, Italy.
- Ciarcia Gaetano, dept. of Biology and Interdept. Research Center for Environment (I.R.C.Env.), Federico II University, Naples, Italy. E-mail: [ciarcia@unina.it](mailto:ciarcia@unina.it)
- Guerriero Giulia, dept of Biology and Interdept. Research Center for Environment (I.R.C.Env.), Federico II University, Naples, Italy. E-mail: [giulia.guerriero@unina.it](mailto:giulia.guerriero@unina.it)

the river and the water quality of the Gulf of Naples [17]. The Sarno River is affected by an extreme environmental degradation as a result of uncontrolled agricultural activities and outflow of industrial waste. Changes in the abundance and diversity of frogs can be used as a measuring tool to determine water quality of Sarno River. The aim of this study is to monitor changes in water quality, sediment and frog tissues and to demonstrate the potential in using frogs as environmental indicators in the development area of Sarno River.

## 2 SITE CHARACTERISTICS

The area has a Mediterranean climate regime, with average annual temperature of 17.2°C and average annual rainfall of 1203 mm, mostly concentrated at the end of the summer [12]. Naples urban territory lies on the eastern flank of the active Phlegrean Fields volcanic district. Its geological structure results from the interplay of several processes: volcanism, tectonics and volcanotectonics, sedimentary, while its coastal and nearshore location favour the influence of the eustatic variations of the sea level [16]. Specifically, reliefs of Sarno basin which cover 440 km<sup>2</sup> are constituted by Triassic dolomite, by dolomitic limestone of the lower Jurassic-Cretaceous as well as by Cretaceous fractured and karstified limestone [18]. The headwater of the Sarno River is located close to the homonymous town at the base of a calcareous formations of the Campanian Apennin Mountains [17]. The river has a daily average flow of about 61 m<sup>3</sup>hr<sup>-1</sup> and a relatively short straight course (24 km) crossing the heavily farmed land of San Marzano and Scafati before flowing into the Tyrrhenian Sea [17]. The Sarno river region is characterized by a series of carbonate rock hills hosting highly differentiated benthic communities. Photophilic associations with dominance of brown algae (*Padina pavonica* and *Sargassum* sp.) dominate the top of hills, while macroalgal assemblages typical of sciaphilic environments (*Peyssonellia borneti* and *Flabellina petiolata*) characterize the vertical walls. A lot of benthic animals are present and typically several species of *Pelophylax* frogs [19].

## 3 MATERIAL AND METHODS

### 3.1 Sampling techniques

Sites were chosen to minimize the influence of point source inputs from sewage treatment facilities. So, a total of seven sampling sites were monitored for water, sediment and soil quality along the Sarno river. Site 1 was located 4 km downstream of the headwaters, upstream of the Nocera entry point which receives the Solofrana and Cavaiola tributaries in the upper Nocera Inferiore valley. Station 2 was downstream near the Nocera confluence. Stations 3-5 were near the towns of Scafati, Pompei and Castellamare, respectively, and Stations 6 and 7 were 1500 and 400 m, respectively, upstream of the mouth. Surface water and sediment samples were taken from the midstream section of the river at 0.25 m below the surface on September 2013 and March 2014. Frogs samples was taken from Scafati and Sarno, and Matese, as control area.

### 3.2 Chemical and physical analyses

The pH, electrical conductivity (E.C.), temperature and dissolved oxygen were determined in situ by a multiparametric probe (Ocean Seven mod 401). Water to be used for laboratory analysis was filtered on site (0.45 µm, single use Sartorius ester cellulose filters). The samples of water, sediment and soil conserved in sterile phials, were obviously labelled for each station on field and then placed in the icebox. Brought to the EC Laboratory of the University Federico II, and stored at -20°C. The decision of using an amphibian like the "green frog" *Pelophylax bergeri* was influenced by the fact that amphibians have a very important role in the actual research on bio-indicators, this is confirmed by the high degree of utilization in numerous scientific studies (physiological study, ecotoxicological study, etc.) [14], [15]. The samples analyzed for heavy metals determination were of 4 different natures:

- Water samples
- Sediment samples
- Soil samples
- Tissues and organs samples.

All samples were analyzed for concentrations of the main metals with the same instrument using mass spectrometry: ICP-QMS Bruker 820-MS. This instrument enables to determine concentrations of heavy metals in every type of sample with previous different pre-analysis treating for every different sample. The treatment necessary for every type of sample will be briefly described for more information on the instrument and its full potentialities can be found in the book by Johanna Sabine Becker "Inorganic Mass Spectrometry, principles and application".

#### 3.2.1 Water samples

Water samples were filtered through a cellulose filter (0,45µm) and kept in the dark at 20°C until analysis. Dissolved phase refers to the fraction of contaminants passing through the filter, and there are both dissolved compounds that those associated with colloidal organic matter. Filtrates were collected and kept at 4°C for the extraction.

#### 3.2.2 Soil and sediment samples

This method is not a total digestion technique for most samples. It is a very strong acid digestion that will dissolve almost all elements that could become "environmentally available". By design, elements bound in silicate structures are not normally dissolved by this procedure as they are not usually mobile in the environment. For the digestion of samples, a representative 1-2 gram (wet weight) sample is digested with repeated additions of nitric acid (HNO<sub>3</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) hydrochloric acid (HCl) is added to the initial digestate and the sample is refluxed. In an optional step to increase the solubility of some metals this digested is filtered and the filter paper and residues are rinsed, with hot HCl and then hot reagent water. Filter paper and residue are then returned to the digestion flask, refluxed with additional HCl and then filtered again. The digested is then diluted to a final volume of 100 ml [20].

### 3.2.3 Tissue and organs samples

Tissues to analyze were selected on the base of the level of exposure to heavy metals presence and ill-omened implications. These tissues were then extracted from the individual. Digestion was carried out with a total of 15 ml of Optima-grade Nitric acid (HNO<sub>3</sub>), the sample (wet, as received) heated and reduced to dryness was reconstituted with and 9 ml of 30% Optima H<sub>2</sub>O<sub>2</sub>, heated and reduced to dryness again and then was reconstituted in 10 ml with 2% Nitric Acid (both Optima-grade)[21].

### 3.3 Morphological and molecular analysis

The frogs once captured were stored in a bucket with a pierced lid to let air through. The buckets were then also brought to the EC Laboratory. Animals were initially morphologically identified by the classical taxonomic keys [22], and all resulted belonging to the genus *Pelophylax*; in particular the species are: *lessonae*, *ridibundus*, *esculenta* and *bergeri*. Samples whose identification was difficult were subjected to the bar-coding molecular investigation.

#### 3.3.1 DNA extraction from frog tissue

The DNA extraction from skin and gonads was performed as reported in [23] with a phenol/chloroform standard method by using autoclaved glassware and equipment. About 50 mg of ground freeze dried tissues were mixed in a DNA extraction buffer (50 mMNaCl, 10 mM EDTA and 10 mMTris base) and the cells were lysed by adding 2% sodium dodecyl sulfate. The RNA was removed by adding RNase (10 mg/mL) followed by incubation at 37°C for 30min. Proteinase K was added (0,5 mg/mL) to remove protein and the samples were incubated for 1h 37°C in a shaking water bath. The extracts were further purified by extracting twice with phenol:chloroform:isoamyl alcohol (25:24:1 v/v) and by centrifuging at 10,000×g for 15 min at 4°C. The upper aqueous layer was transferred into a new micro-centrifuge tube and the DNA was precipitate by adding 1/10th volume of 3 M sodium acetate at pH 5.2 and two volumes of 100% chilled ethanol to each sample and mixed centrifuged at 15,000×g for 30 min at 4°C. The pellet was washed with 70% ethanol, air dried, and finally re-suspended in 50 µl sterilized deionized water. Optical density (OD) of each sample was measured at 260 and 280 nm respectively, by UV-spectrophotometer (Biochrom Libra S12), and the purity of DNA was measured by the OD260/OD280 ratio (ideal ratio = 1.7-2.0), and the quality by electrophoresis on a 0,8% agarose gel and visualized under UV light[23].

#### 3.3.2 PCR amplification and sequencing of 16S mt-rRNA gene fragment

PCR amplification was performed as previously published (Guerriero et al. 2010) using the following primers: 5\_-CGCCGTGTTATCAAAAACAT -3\_ (16Sar) and 5\_-CCGGTCTGAACTCAGATCACGT- 3\_ (16Sbr) for 16S rRNA gene (Palumbo et al., 1996). PCR reaction was performed in a Techgene Thermal Cycler (Thecne Ltd., Cambridge, UK). Thir-

ty-five cycles of amplification were carried out in a reaction buffer containing 50 mM KCl, 10 mM Tris/HCl, pH 9.0; 10 mM NaCl; 0.01 mM EDTA; 2.5 mM of each dNTP; 1 µM of each primer; 10 ng of template DNA; 0.5 unit of Taq DNA polymerase (Invitrogen, Milan, Italy). PCR amplification conditions were as follows: denaturation at 94°C for 45 s, annealing at 55°C for 50 s, and extension at 72°C. for 90 s. At the end of the incubation, 5 µl of PCR products were separated by electrophoresis through 2% agarose gel and visualized under UV light. A 100 bp ladder (Invitrogen, Milan, Italy, or Fermentas, M-Medical srl, Milan, Italy) was used to estimate the fragment size of the amplicons generated. Amplified DNA was desalted with Microcon 100 spin columns (Millipore-Amicon, Belford, MA, USA) according to the manufacturer's instructions and sequenced using Big Dye TM Terminator Cycle Sequencing Chemistry (Applied Biosystems, Foster City, CA, USA) in an automatic capillarity sequencer (ABI 310 Genetic Analyzer; Applied Biosystems). Primers for sequencing were the same used for PCR amplification [24], [25].

#### 3.3.3 Sequence analysis

Resulted sequences were analyzed and aligned using Chromas 1.45 vs (Technelysium 186 Pty, Tewantin, Australia) and BioEdit (Tom Hall Ibis Therapeutics, Rutherford Road Carlsbad, CA) software and compared with GenBank sequences using FASTA (Mount, 2007, FASTA SIMILARITY SEARCH) [26].

## 4 ANALYSIS, RESULTS AND DISCUSSION

### 4.1 In situ parameters

#### a. Air Humidity (A.H) and Air temperature (A.T)

In the study site, Sarno river, air humidity in wet period ranges from 43 to 84 percent with a mean value about 62 %. In the same period air temperature ranges from 8 to 15 °C with average value as 11 °C (Table 01).

#### b. Water temperature (W.T)

In dry period (September 2013) water temperature ranges from 12 to 25 °C (Table 01) with a mean about 11 °C but in a wet period (March 2014) it ranges from 12 to 15 °C with an average value as 13.5 °C. In general and in all periods the temperature in Sarno river is not exceeding standards (Table 01).

Table 01 Variation of air humidity, air temperature, and water temperature in dry period (September 2013) and wet period (March 2014) of Sarno River.

Parameters	A. H %		A. T °c		Water T °c		
	2013	2014	2013	2014	2013	2014	
Statistical Parameters	Max.	/	84	/	15	24.46	14.98
	Mean	/	62	/	11	19.06	13.51
	Min.	/	43	/	8	12.57	12.28
	Std.Dev	/	15	/	2	4.81	0.91
Standards	/		/		< 25 [27]		

**c. Potential of hydrogen (pH).**

In dry period (September 2013) potential of hydrogen (pH) ranges from 5.69 to 7.31 (Table 02) with a mean about 6.58 but in a wet period (March 2014) it ranges from 6.10 to 7.07 with an average value as 6.55. In general and in all periods the potential of hydrogen in Sarno river sometimes is exceeding standards (Table 02) as an acid environment (Fig.01).

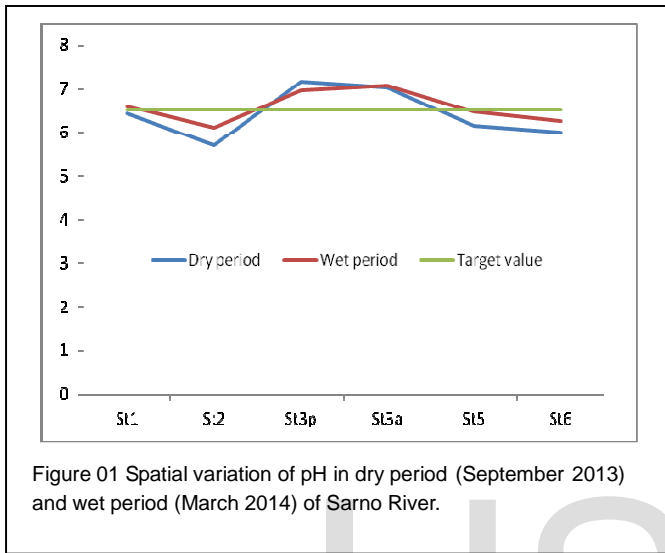


Figure 01 Spatial variation of pH in dry period (September 2013) and wet period (March 2014) of Sarno River.

**d. Salinity**

In dry period (September 2013) salinity ranges from 0.41 to 38 ppt (Table 02, Fig. 01) with a mean about 0.56 ppt but in a wet period (March 2014) it ranges from 0.32 to 0.62 ppt with an average value as 0.48 ppt (Table 02). In general and in dry period the salinity at downstream Sarno river is sometimes very high (Fig. 02) especially in points which are close to sea (St7.1, St7.2).

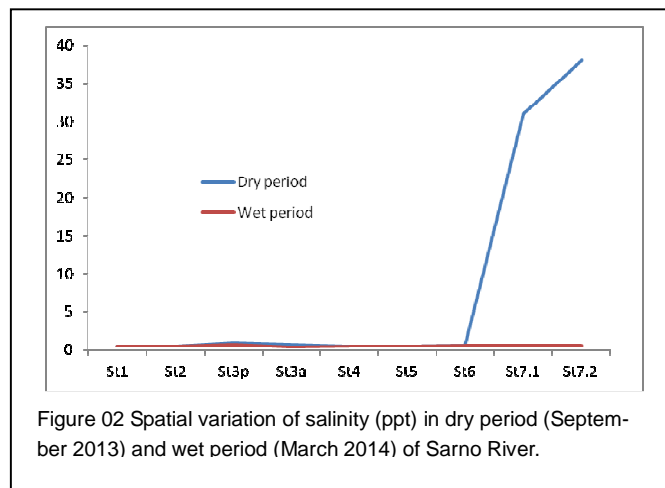


Figure 02 Spatial variation of salinity (ppt) in dry period (September 2013) and wet period (March 2014) of Sarno River.

**e. Dissolved oxygen (DO)**

In dry period (September 2013) dissolved oxygen ranges from 3 to 16 mg/l (Table02) with a mean about 9 mg/l but in a wet

period (March 2014) it ranges from 9 to 17 mg/l with an average value as 15mg/l. In general dissolved oxygen doesn't exceed standards (Table 02, Fig.03) only in dry period especially in one point (St1) which is situated in the upriver of Sarno.

Table 02. Variation of pH, Salinity and Dissolved Oxygen in dry period (September 2013) and wet period (March 2014) at Sarno River.

Parameters	pH		Salinity ppt		D.O mg/l		D.O %		
	2013	2014	2013	2014	2013	2014	2013	2014	
Statistical Parameters	Max.	7.31	7.07	38	0.62	16.20	17.25	178	161
	Mean	6.58	6.55	0.56	0.48	9.33	14.69	113	141
	Min.	5.69	6.10	0.41	0.32	3.23	9.90	39	98
	Std.D ev.	0.60	0.35	0.16	0.08	4.74	2.38	56	20
Standards	6.5-9.5 [27]		/		> 5 [28]		> 50 [28]		

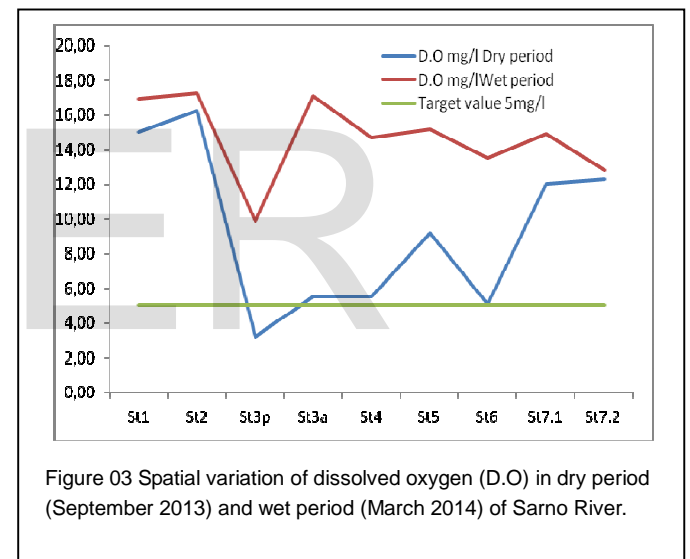


Figure 03 Spatial variation of dissolved oxygen (D.O) in dry period (September 2013) and wet period (March 2014) of Sarno River.

**4.2 Frog identification**

DNA extracted from all examined skin and gonads of sampled frogs constitutes a more efficient template, indicating a good yield of PCR products. The described set of primers successfully have amplified the mitochondrial region fragments examined, of approximately 630bp long (data not shown). The PCR products isolated from gel, sequenced and aligned with GenBank (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov) sequences data for 12S rRNA of *Pelophylax bergeri*, Günther, 1986 (accession number AJ222650), *ridibundus*, Pallas, 1771 (accession number AB640897), *lessonae*, Camerano, 1882 (accession number AB023395) using FASTA have confirmed the morphological identification and allowed the selection of *P. bergeri* samples. Furthermore, sequences information for 16S rRNA genes for *Pelophylax bergeri* was not previously published in the literature.

**4.3 Metal parameters**

**4.3.1 Non Toxic metals**

**Cobalt (Co)**

Cobalt content in Sarno river water (table 03) ranges from 0.60 to 4.13 with 2.61 ug/l as average value. The highest concentrations are in soil than sediment and range from 2 to 4 mg/kg. Cobalt content in frogs is higher in gonad than skin and ranges from 0.77 to 11.06 mg/kg.

Table 03 Variation of the Cobalt (Co) at Sarno River (2013).

Parameters		Water µg/l	Sediment mg/kg	Soil mg/kg	Frog (mg/kg)	
					Skin	Gonad
Statistical Parameters	Max.	4.13	4	4	0.15	11.06
	Mean	2.61	1.69	3	0.11	7.21
	Min.	0.60	0.13	2	0.08	0.77
	Std.Dev.	1.18	1	0.6	0.03	5.60
Standards		/				

**Manganese (Mn)**

Manganese content in Sarno river water (table 04) ranges from 0.10 to 168 with 43 ug/l as mean value. The highest concentration is downstream of Sarno river exceeding standards (50ug/l) especially in St3p and St5 stations (Fig.04). Manganese concentrations are higher in soil than sediment and range from 51 to 133 mg/kg. Manganese content in frogs is higher in gonad than skin and ranges from 8 to 47 mg/kg.

Table 04 Variation of the Manganese (Mn) at Sarno River (2013).

Parameters		Water µg/l	Sediment mg/kg	Soil mg/kg	Frog (mg/kg)	
					Skin	Gonad
Statistical Parameters	Max.	168	81	133	10	47
	Mean	43	58	81	7	32
	Min.	0.10	31	51	2	8
	Std.Dev.	60.8	18	33	4.00	21
Standards		50 µg/l [27] /				

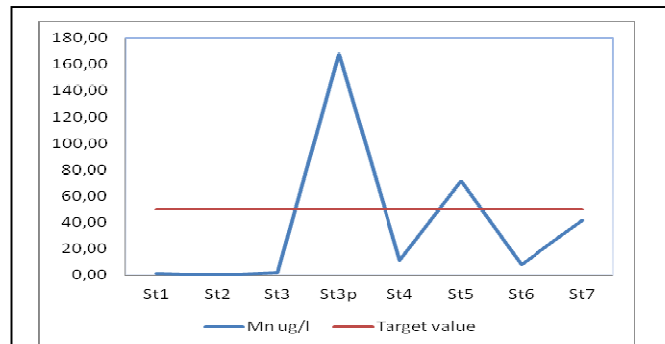


Figure 04 Spatial variation of the Manganese (Mn) in water Sarno River (2013).

**Molybdenum (Mo)**

Molybdenum content in Sarno river water (table 05) ranges from 0.21 to 0.69 with 0.42 ug/l as average value. The soil and sediment concentrations are very low and not exceeding 0.15 mg/kg. Molybdenum content is also very low in frogs and is higher in gonad than skin and ranges from 0.49 to 0.97 mg/kg (table05).

Table 05 Variation of the Molybdenum (Mo) at Sarno River (2013).

Parameters		Water µg/l	Sediment mg/kg	Soil mg/kg	Frog (mg/kg)	
					Skin	Gonad
Statistical Parameters	Max.	0.69	0.150	0.082	0.015	0.97
	Mean	0.42	0.081	0.050	0.012	0.64
	Min.	0.16	0.049	0.020	0.009	0.07
	Std.Dev.	0.21	0.040	0.027	0.003	0.49
Standards		/				

**Selenium (Se)**

Selenium content in Sarno river water (table 06) ranges from 1.93 to 6.54 with 4.38 ug/l as average value. The soil and sediment selenium concentrations are very low and not exceeding 0.73 mg/kg. Selenium content is also very low in frogs and is higher in gonad than skin and ranges from 19.45 to 36.77 mg/kg (table06).

Table 06 Variation of the Selenium (Se) at Sarno River (2013).

Parameters		Water µg/l	Sediment mg/kg	Soil mg/kg	Frog (mg/kg)	
					Skin	Gonad
Statistical Parameters	Max.	6.54	0.52	0.73	0.78	36.77
	Mean	4.38	0.32	0.53	0.63	23.62
	Min.	0.32	0.05	0.24	0.45	1.27
	Std.Dev.	1.93	0.21	0.19	0.16	19.45
Standards		/				

**Iron (Fe)**

Iron content in Sarno river water (table 07) is very low and ranges from 2 to 5 with 5 ug/l as mean value not exceeding standards in all stations. The highest concentrations are in soil than sediment and range from 400 to 5000 mg/kg. Frogs iron content is higher in gonad than skin and ranges from 3800 to 7400 mg/kg.

Table 07 Variation of the Iron (Fe) at Sarno River (2013).

Parameters		Water µg/l	Sediment mg/kg	Soil mg/kg	Frog (mg/kg)	
					Skin	Gonad
Statistical Parameters	Max.	7	3707	4917	83	7398
	Mean	5	3093	4428	66	4626
	Min.	2	2208	3864	47	264
	Std.Dev.	1.65	581	400	17	3823
Standards		200 µg/l [27] /				

**4.3.2 Toxic metals**  
**Arsenic (As)**

The table 08 shows an increase between 2008 and 2013 in the arsenic content in Sarno river water. So, it ranges in 2013 from 16 to 2999 with 450 ug/l as mean value and greatly exceeding the standards in all stations along the Sarno river (Fig.05). Arsenic concentrations in soil and sediment are very low and not exceeding standards in comparison with gonad frogs which are very high and exceeding the soil arsenic standards. Arsenic concentrations are higher in gonad than skin and ranges from 49 to 327 mg/kg.

Table 08 Variation of the Arsenic (As) at Sarno River (2013).

Parameters		Water $\mu\text{g/l}$			Sediment $\text{mg/kg}$ 2013	Soil $\text{mg/kg}$ 2013	Frog (mg/kg) 2013	
		2008	2009	2013			Skin	Gonad
Statistical Parameters	Max.	12.40	3.60	2999	3.40	4.27	4.96	327
	Mean	4.13	2.98	450	2.62	3.46	2.82	224
	Min.	1.6	1.9	16	1.70	3.02	1.60	49
	Std.Dev.	2.93	0.58	1124	0.70	0.50	1.80	152
Standards		10 $\mu\text{g/l}$ [27]			30 $\text{mg/kg}$ [29]; [30]			

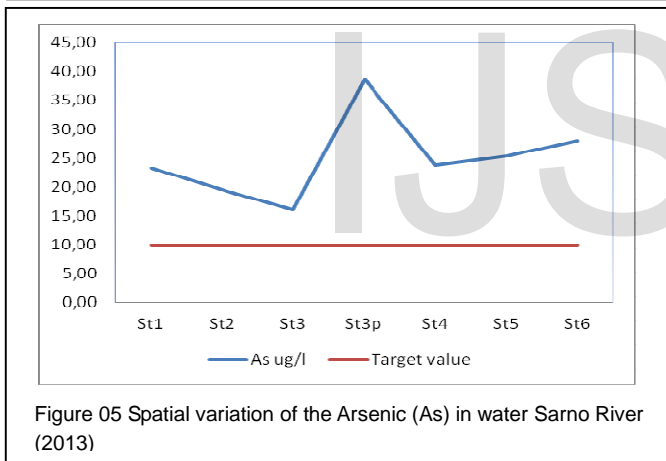


Figure 05 Spatial variation of the Arsenic (As) in water Sarno River (2013)

**Cadmium (Cd)**

Cadmium content in Sarno river water (table 09) is very low and not exceeding standards in all stations and in all years and also in sediment and soil.

Table 09 Variation of the Cadmium (Cd) at Sarno River (2013).

Parameters		Water $\mu\text{g/l}$			Sediment $\text{mg/kg}$ 2013	Soil $\text{mg/kg}$ 2013	Frog (mg/kg) 2013	
		2008	2009	2013			Skin	Gonad
Statistical Parameters	Max.	0.20	0.20	1.05	0.20	0.322	/	/
	Mean	0.11	0.13	0.54	0.15	0.224	/	/
	Min.	0.01	0.10	0.14	0.09	0.180	/	/
	Std.Dev.	0.04	0.04	0.33	0.04	0.056	/	/
Standards		5 $\mu\text{g/l}$ [27]			1 $\text{mg/kg}$ [29]; [30]			

**Chromium (Cr)**

The table 10 shows a decrease between 2008 and 2013 the chromium content in Sarno river water. So, it ranges in 2013 from 12 to 35 with 8 ug/l as mean value and not exceeding the standards in all stations along the Sarno river (Fig.06). Chromium concentrations in soil and sediment are very low and not exceeding standards in comparison with gonad frogs which are very high and exceeding the soil chromium standards. Chromium concentrations are higher in gonad than skin and ranges from 39 to 77 mg/kg almost exceeding standards of contaminated soil.

Table 10 Variation of the Chromium (Cr) at Sarno River (2013).

Parameters		Water $\mu\text{g/l}$			Sediment $\text{mg/kg}$ 2013	Soil $\text{mg/kg}$ 2013	Frog (mg/kg) 2013	
		2008	2009	2013			Skin	Gonad
Statistical Parameters	Max.	344	44	35	10.03	29.85	0.32	77
	Mean	42.40	17.17	8	4.23	19.84	0.28	48
	Min.	0.10	0.40	0.22	0.95	5.23	0.24	3
	Std.Dev.	87.78	15.74	12	3.57	13.05	0.03	39
Standards		50 $\mu\text{g/l}$ [27]			60 $\text{mg/kg}$ [29]; [30]			

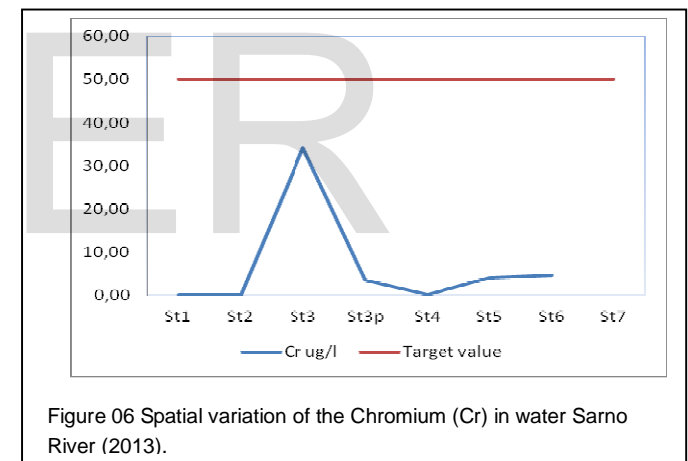


Figure 06 Spatial variation of the Chromium (Cr) in water Sarno River (2013).

**Nickel (Ni)**

Nickel content in Sarno river water (table 11) is very low and not exceeding standards in all stations and in all years and also in sediment and soil.

Table 11 Variation of the Nickel (Ni) at Sarno River (2013).

Parameters		Water $\mu\text{g/l}$			Sediment $\text{mg/kg}$ 2013	Soil $\text{mg/kg}$ 2013	Frog (mg/kg) 2013	
		2008	2009	2013			Skin	Gonad
Statistical Parameters	Max.	8.40	3.00	9.58	7.16	9.53	/	/
	Mean	2.44	2.28	5.25	4.53	7.69	/	/
	Min.	0.20	1.20	0.99	0.16	6.01	/	/
	Std.Dev.	1.80	0.50	2.53	2.70	1.59	/	/
Standards		20 $\mu\text{g/l}$ [27]			30 $\text{mg/kg}$ [29]; [30]			

### Lead (Pb)

The table 12 shows a decrease between 2008 and 2013 of the lead content in Sarno river water. So, it ranges in 2013 from 0.2 to 0.4 with 0.22 ug/l as mean value and not exceeding the standards in all stations along the Sarno river. Lead concentrations in soil and sediment are very low and not exceeding standards (table 12).

Table 12 Variation of the Lead (Pb) at Sarno River (2013).

Parameters	Water µg/l			Sediment mg/kg 2013	Soil mg/kg 2013	Frog (mg/kg) 2013		
	2008	2009	2013			Skin	Gonad	
Statistical Parameters	Max.	19.10	5.00	0.40	14	25	/	/
	Mean	2.32	2.41	0.22	12	19	/	/
	Min.	0.10	0.60	0.05	8	14	/	/
	Std.Dev.	4.19	1.27	0.2	2	5	/	/
Standards	10 µg/l [27]			50 mg/kg [29][30]				

### Copper (Cu)

The table 13 shows an increase of copper content in Sarno river water between 2008 and 2013. So, it ranges in 2013 from 5 to 69 with 65 ug/l as mean value and not exceeding the standards in all stations along the Sarno river. Copper concentrations in soil and sediment are almost high and exceeding sometimes standards but in skin of frogs are very low.

Table 13 Variation of the Copper (Cu) at Sarno River (2013).

Parameters	Water µg/l			Sediment mg/kg 2013	Soil mg/kg 2013	Frog (mg/kg) 2013		
	2008	2009	2013			Skin	Gonad	
Statistical Parameters	Max.	31.40	16.00	69	63	113	2	/
	Mean	6.53	8.33	65	31	38	0.6	/
	Min.	0.60	3.30	60	14	15	0.09	/
	Std.Dev.	6.81	3.81	5	19	42	0.8	/
Standards	1000 µg/l [27]			50 mg/kg [29][30]				

### Mercury (Hg)

The table 14 shows an increase of Mercury content in Sarno river water between 2008 and 2013. So, it ranges in 2013 from 0.1 to 6 with 1.2 ug/l as mean value and greatly exceeding the standards downstream the Sarno river (St7). Mercury concentrations in soil and sediment are very low and not exceeding standards.

Table 14 Variation of the Mercury (Hg) at Sarno River (2013).

Parameters	Water µg/l			Sediment mg/kg 2013	Soil mg/kg 2013	Frog (mg/kg) 2013		
	2008	2009	2013			Skin	Gonad	
Statistical Parameters	Max.	0.80	5.70	6	0.06	0.11	/	/
	Mean	0.33	1.13	1.2	0.05	0.09	/	/
	Min.	0.10	0.30	0.1	0.04	0.08	/	/
	Std.Dev.	0.12	1.89	2.2	0.008	0.01	/	/
Standards	1 µg/l [27]			1 mg/kg [29][30]				

### Zinc (Zn)

The table 15 shows an increase between 2008 and 2013 of the zinc content in Sarno river water. So, it ranges in 2013 from 73 to 115 with 63 ug/l as mean value and not exceeding standards in all stations along the Sarno river. Zinc concentrations in soil and sediment are very low and not exceeding standards in comparison with gonad frogs which are very high and almost exceeding the soil zinc standards. Zinc concentrations are higher in gonad than skin and ranges from 181 to 366 mg/kg exceeding contaminated soil target value.

Table 15 Variation of the Zinc (Zn) at Sarno River (2013).

Parameters	Water µg/l			Sediment mg/kg 2013	Soil mg/kg 2013	Frog (mg/kg) 2013		
	2008	2009	2013			Skin	Gonad	
Statistical Parameters	Max.	70	80	115	89	85	73	366
	Mean	17	40	63	63	54	57	243
	Min.	1	19	11	37	37	38	35
	Std.Dev.	16	35	73	20	19	17	181
Standards	3000 µg/l [27]			120 mg/kg [29][30]				

## 5 CONCLUSION

In summary, we briefly reviewed the Sarno river site characteristics and problematic and the general advantages and limits of the chemical and biological monitoring methods, hence the degree of metals pollution in the Sarno river detection through the year 2013 and 2014. The metals concentration varies by zone, as well as by metal to metal, contents are often very elevated in frogs and higher in water and soil than sediment. Results show that in the frog specie *bergeri* of the genus *Pelophylax*, the more representative in Sarno River, metals content varies inter and not intra tissue. The concentrations of heavy metals, especially Arsenic (As) Chromium (Cr) and Zinc (Zn) were found higher in testis than in skin of the frogs. The frog gonad is thus an interesting candidate for further studies as bioindicator and the metals detection on it a valid biomarker for the river health evaluation and the biodiversity preservation assessment. Beside the major contribution of the *Pelophylax bergeri* 16 S mt-rRNA sequence, submitted to Genbank, could permit to follow its pro site abundance index. Furthermore frogs, as water of Sarno River, are very contaminated by arsenic and an urgent remediation for the entire ecosystem health is requested.

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