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RESEARCH ARTICLE

Heavy Metals Assessment in the Medjerda River Basin (Northeastern Algeria): A Preliminary Water Analysis and Toad Skin Biopsy

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Abstract Our study attempted to monitor the quality of water in Medjarda basin (Northeastern Algeria) and to provide baseline information of heavy metals in the water as well as in a potential amphibian biosentinel, the spiny toad, *Bufo spinosus*. We measured pH, temperature, dissolved oxygen and biological oxygen demand of water and levels of heavy metals in toad skin using an atomic absorption flame spectrophotometer. Lead (Pb) concentration in water and in toad skin at all sites exceeded respectively 60 and 96 times the standard reference values. The heavy metal concentrations, in descending order, in water and in male toad skin were as follows: Pb > Fe > Cu > Zn and Fe > Pb > Zn > Cu respectively. This study highlights the ecological status of the surrounding areas upstream of the Medjarda basin as being a point source of heavy metal pollution. It is further stated that a non-invasive skin removal is an ethically sound technique to evaluate heavy metal accumulation in aquatic animals like toad, without euthanizing the specimens and making any loss to biodiversity of the species.

Keywords Heavy metal · Medjarda basin · Spiny toad · Skin biopsy · Non-invasive methods

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Introduction

The quality of groundwater and surface water in rural and urban environments is influenced by natural and anthropogenic processes (Tang et al. 2014; Fasulo et al. 2015; Lecomte et al. 2017; Vardhan et al. 2019). With anthropogenic human activities, there has been a significant increase in the discharge of industrial waste into the environment, especially in urban areas, which creates an alarming situation for human life and aquatic biota (Jan et al. 2015; Lecomte et al. 2017; Guerriero et al. 2019). The anthropogenic factors include discharge of organic pollutants such as drugs, personal care products, steroids, hormones, endocrine disruptors, surfactants, phosphoric esters, flame retardants, industrial additives and siloxanes into natural freshwater bodies (Jaouadi et al. 2012; Khatri and Tyagi 2015; Lecomte et al. 2017; Parisi and Guerriero 2019). Both natural and the anthropogenic processes alter the heavy metals, coliforms and nutrient load concentrations in these water bodies. As a result of uncontrolled agriculture, unplanned urbanization and industrialization rivers have been reported to be polluted with heavy metals in many countries (Adekola and Eletta 2007; Mohiuddin et al. 2011; Ali et al. 2016; Pandey and Singh 2017). Several metals essential for life are naturally found in water but their levels must be under strict assessment because a concentration higher than the acceptable range may be toxic to aquatic organisms (El-Kady and Abdel-Wahhab

2018; Diaconu et al. 2020). Such assessment can avoid a serious impact on the global biodiversity (Ficken and Byrne 2013; Jan et al. 2015; Guerriero et al. 2018a). Many regions in Algeria have been developed without adequate planning resulting in soils and water reported as being heavily polluted with various metals including mercury (Hg), arsenic (As), lead (Pb), zinc (Zn), manganese (Mn), lithium (Li), cadmium (Cd) and chromium (Cr) (Tahar and Keltoum 2011; Benhaddya and Hadjel 2014; Larba and Soltani 2014; Boudia et al. 2019; Talbi and Kachi 2019). Evidence of different metals also appears in the waters of Medjarda River, an urban river of Souk-Ahras City in Northeastern Algeria. However, as per current literature there is no document on heavy metal contamination in the Medjarda basin.

Environmental programs in the last decades highlight the importance of organisms for monitoring climate changes and pollutants (Mani and Kumar 2014; De Maio et al. 2014; Dixit et al. 2015; Guerriero et al. 2018a,b; Gentilucci et al. 2020). Aquatic animals such as amphibians can accumulate heavy metals from the environment directly by absorption through skin and oral surfaces and indirectly by ingestion of heavy metals contaminated food items. Heavy metal toxicity to organisms can damage the function of various organs. This is especially true for nonessential elements, such as Pb, which is highly toxic even at low concentrations (Niethammer et al. 1985; Jaishankar et al. 2014; Ben Hassine and Escorzia 2017).

The documented effects of pollutants on biosentinels, such as amphibians, range from lethal to sublethal effects and include decreased growth and fertility, increased frequency of developmental abnormalities, increased susceptibility to disease, behavioral changes, and damage to germ lines (Ortiz et al. 2004; Karraker et al. 2008; Shinn et al. 2008; Snodgrass et al. 2008; Guerriero et al. 2009; Guerriero et al. 2011; Guerriero et al. 2019; Parisi and Guerriero 2019). Furthermore, due to the feeding habits of larval microphages of most species, toads frequently ingest sediments through which metals can be accumulated (Hopkins and Rowe 2010; Burlibaşa and Gavrilă 2011; James and Semlitsch 2011).

Several researchers have attempted to document the impacts of metal buildup in amphibians both in captivity (Herkovits and Helguero 1998; James and Little 2003; D'Errico et al. 2018; Guerriero et al. 2018b; Pinelli et al. 2019), and in field conditions (Demichelis et al. 2001; Borkin and Flyaks 2004; Fenoglio et al. 2006; Gavrilović et al. 2020). Other studies have reported deformity, delay in metamorphosis, reduced flight response and altered interactions with predators in amphibians after exposure to metals in water (Chen et al. 2009; James and Semlitsch 2011; Zocche et al. 2013; Chagas et al. 2020). Furthermore, heavy metal assessment using amphibians along the most polluted river in Europe demonstrated the useful indication provided by the different tissues in these organisms (Trocchia et al. 2015). For all of these reasons, amphibians can be used as sensitive bio-indicators in monitoring effects of heavy metals and other anthropogenic activities in aquatic environment (Prokić et al. 2016; D'Errico et al. 2018; Guerriero et al. 2018b). Use of a non-invasive skin biopsy is a more relevant bio-monitoring process, because it does not require euthanizing of the animal specimens and thus avoids ethical issues. The non-invasive procedure can be applied on large numbers of specimens and also can be used for repeated assessments without negatively impacting the population of the selected amphibian species (D'Errico et al. 2018).

In order to study the state of pollution propagation and contamination of heavy metal in the environment, a potential biosentinel was chosen as representative of ecological damage. The spiny toad *Bufo spinosus* (Amphibia: Anura) was chosen for this purpose because, it is present in Algeria and in the wettest and most temperate zones in North Africa (Tlidjane et al. 2019). The choice of an amphibian was based on their key ecological role in the trophic networks as prey or predators in a wide range of ecosystems (Niethammer et al. 1985; Ben Hassine and Escorzia 2017; Pinelli et al. 2019). In fact, frogs usually use their own skin as a secondary respiratory surface, which is permeable to endogenous and exogenous substances whose potential harmful effects are contrasted by keratins. This factor makes them suitable as a biosentinel (Guerriero et al. 2018b, c) to assess environmental contamination. In this study, *Bufo spinosus* underwent a non-invasive skin removal procedure.This procedure, already successfully tested on Italian pool frog *Pelophylax bergeri*, exhibited how adverse impact on the environment could be mitigated by avoiding euthanasia of the biosentinel organisms (D'Errico et al. 2018).

Therefore, our research, as part of a larger project on the pollution affecting the Medjarda basin, was designed with the specific aim of contributing to knowledge regarding the distribution of heavy metals (Zn, Cu, Pb and Fe) in the water and in the skin of the most distributed amphibian. The spiny toad, *Bufo spinosus* collected along the river provides baseline information on pollutant distribution as well as determining the point source of heavy metals.

Materials and Methods

Study Area

The study was conducted along the Medjarda River located in Souk-Ahras City in northeastern Algeria (Fig. 1). Covering an area of about 1506 Km^2, the watershed is drained by the Medjerda River and its tributaries. The population is estimated at 340,000 inhabitants, with an estimated growth rate of 1.7%. Located near the eastern edge of Algeria, the Medjerda River is a trans-frontal river, with head waters in the Montagnes region before traversing the urban section. Finally, the river drains to Tunisia, which possibly transports pollutants to different basins downstream. Under the Climate Classification System, the study area has a semi-arid climate characterized by a short dry season.

Sampling Sites

A reference control site (Ctrl) and five sampling sites (1–5) along the river (Fig. 2) were selected for collecting specimens of male toads and water. The sampling sites were chosen according to the variation of anthropogenic activities close to each city between up and downstream; the distances between control and five points were: 2, 7, 15, 27 and 33 km respectively. The Ctrl sampling site (36°, 16′ 35.46″ N 7°45′ 44.60″ E) was located at the mouth of the main river upstream and away from any anthropogenic activities. Sampling site 1 (36°15′ 36.81″N 7°47′ 5.44″ E) was located near H'Nancha city. Sampling sites 2 and 3 (36° 15′ 7.49″ N 7° 47′ 51.30″ E and 36° 15′ 59.59″ N 7° 54′ 44.04″ E) were situated upstream and downstream of Ain Dalia Dam respectively. Site 4 was located close to the Zaarouria village, south of the city of Souk-Ahras (36° 14′ 40.84″ N 7° 57′ 23.36″ E) while site 5 (36° 16′ 14.73″ N 7° 59′ 50.28″ E) was located to the southeast of the same city.

Water Sampling and Analyses

Water from each sampling site was collected along Medjerda River. Water samples were filtered through a cellulose filter (0.45 µm) 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 elements and those associated with colloidal organic matter.

Concentrations of Zn, Cu, Pb and Fe were estimated following the method (Fransion 1981), using an atomic absorption spectrophotometer (Shimadzu AA 6200, Japan). Detection limit of Zn, Cu, Pb and Fe in the instrument was 10 − 3 mg/L, 2 × 10 − 3 mg/L, 8 × 10 − 3 mg/L and 6 × 10 − 3 mg/L, respectively. Appropriate precautions

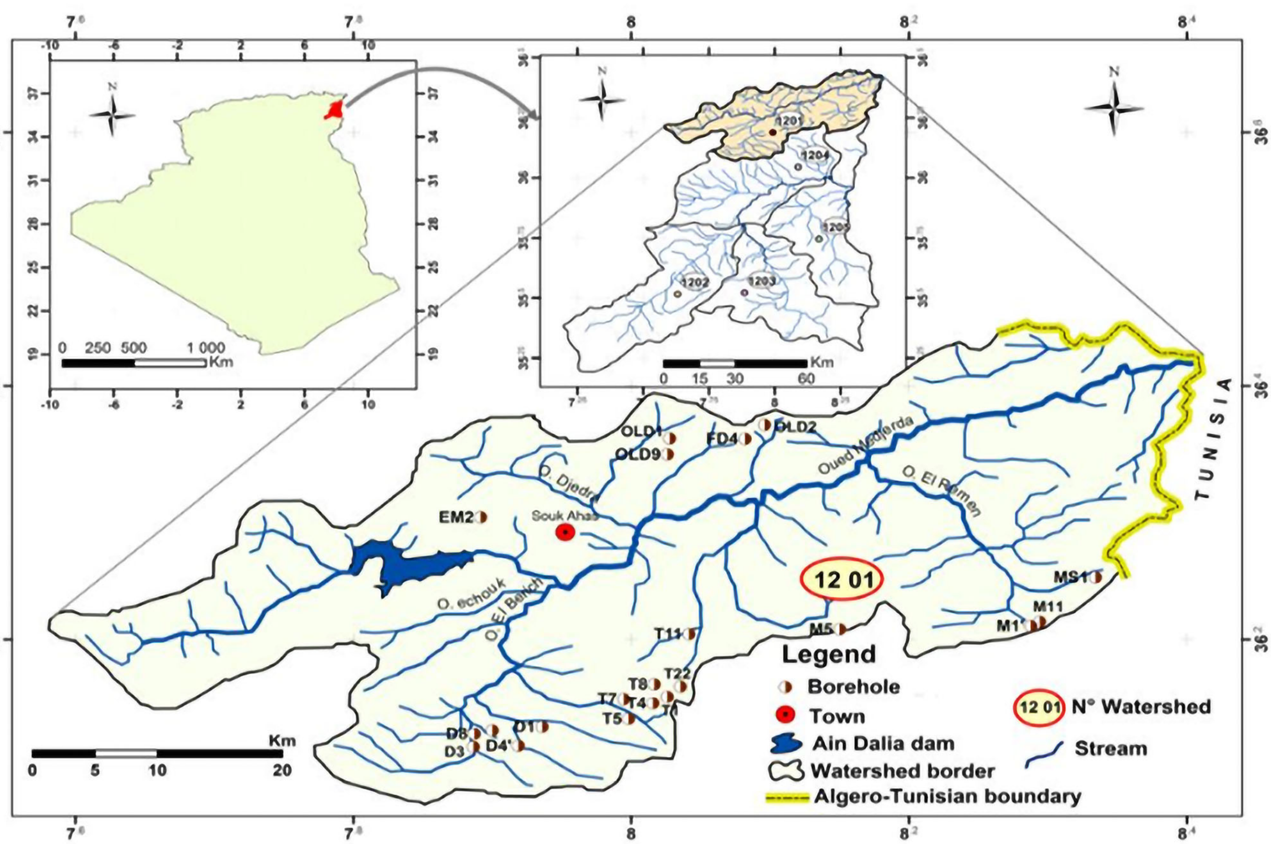

Fig. 1 Geographical location of the Medjerda River basin

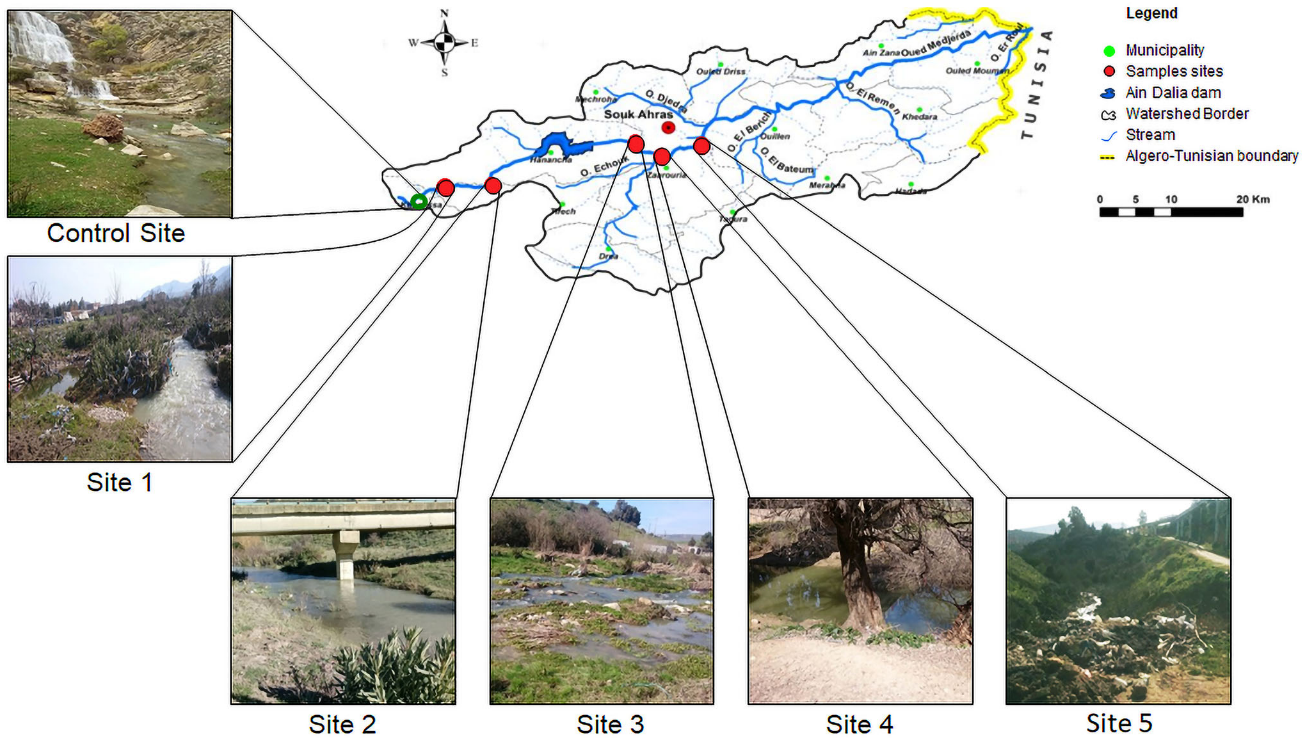

Fig. 2 Sampling sites of water and toads

were taken to maintain precision and accuracy of the determinations.

Chemical-physical parameters of water: pH, temperature, DO (dissolved oxygen) and the BOD (biological oxygen demand) were measured in situ using a multi-parametric probe (Ocean Seven mod 401) at each site during the sampling and was done twice a week in the four weeks of March 2019. The water samples were collected in glass sterilized bottles and were stored with 3% (v/v) conc. HNO_3 acid for heavy metal analysis (G03 Committee).

Animal Sampling and Tissue Processing

Five adult male toads (weight range 150–200 g for each site 2019) identified as *Bufo spinosus* (Daudin 1803), were randomly captured from water ways in the wild twice a week during March 2019 using pitfall traps as standard technique for toad capture (Heyer et al. 1994). After in situ biopsy along the river, the toad samples were released. The non-lethal skin biopsy protocol was performed according to the procedure by D'Errico et al. (2018) by collecting 1 cm^2 skin from each toad. The pool of skin biopsy tissue was dried in an oven at 120 °C for 3 h. Weight of the collected tissue samples were recorded before and after drying. Aliquots of 0.5 g of tissue were placed in 5 ml of nitric acid (69%) and left at room temperature to allow for digestion for 5 days to obtain solution of suspended grease. The samples were then filtered and diluted with deionized water to a final volume of 10 ml. Concentrations of Zn, Cu, Pb and Fe in each sample were estimated using atomic absorption spectrophotometry (Fransion 1981).

Statistical Analysis

The experimental data were analyzed using STATISTICA software (Version 8.0, StatSoft Ltd, USA). Principle component analysis (PCA) and cluster analysis (CA) were used to graphically visualize the hierarchical clustering of the heavy metals in water according to sampling sites. PCA was performed on log-transformed data. CA was performed to classify heavy metals from different pollutant sources on the basis of similarities in their chemical properties. In addition, ANOVA was performed on data of heavy metals of water and toad skin followed by Least Significance Difference (LSD) test to compare significance difference between the study sites. The level of significance was set at $p < 0.05$. The results are presented as mean ± SD.

Results

Physicochemical parameters of water showed no significant differences between the five sampling sites where temperature varied approximately from 11 to 25 °C, and pH (6.9 ± 0.4), dissolved oxygen (DO) (9.5 ± 0.2 ppm) and biological oxygen demand (BOD) (6.8 ± 0.2 ppm) showed no marked variations.

Concentrations of Pb, Fe, Cu and Zn in water of the Medjerda river basin are shown in Fig. 3.The metal concentrations in the five considered sites show two distinct groups in comparison to the control area. A predominant group consisting of Pb and Fe had levels in the range of 0.43–0.72 mg/L and 0.35–0.53 mg/L respectively and an under-represented group consisting of Cu and Zn had levels in the range 0.08–0.12 mg/mL and 0.05–0.11 mg/mL respectively. Specifically Pb, Fe, Cu and Zn concentrations in the five stations didn't significantly differ between sites even though they significantly varied from the control area where Pb, Cu and Zn were undetectable (F value = 0.029; p value = 0.998).

Therefore, based on the mean concentration of Pb, Fe, Cu and Zn (0.60 ± 0.11, 0.43 ± 08, 0.09 ± 0.02 and 0.09 ± 0.03 mg/L, respectively) these heavy metals in water were arranged in the following decreasing order of concentration: Pb > Fe > Cu > Zn.

Since the permissible limits of heavy metals for water quality based on World Health Organization (WHO) established standards are: Pb 0.01 mg/L; Fe 1–3 mg/L; Cu 2 mg/L and Zn 3 mg/L (WHO 2008), the results of the present study reveal values 60 times higher for Pb while the other metals had concentrations within permissible limits.

The results of multivariate statistical analysis, PCA and CA, are shown in Fig. 4. The PCA grouped Pb, Fe, Cu and Zn into a three component model which accounted for 80% of all the data variation. The first principal component (PC1) was correlated with Cu and Fe. The second principal component (PC2) includes Zn only while Pb was unequivocally isolated in the third component (PC3) (Fig. 4a). In fact, Pb and Zn both display rather high values, and they are partially represented in (PC1). The result of CA underlines two distinct clusters which can be identified in cluster I containing Pb and Fe and cluster II containing Zn and Cu as shown in Fig. 4b.

Metals concentration measurements in toad skin biopsy pools (n = 40 males, spiny toad, *Bufo spinosus* each site) were performed to examine Pb, Fe, Zn and Cu (Fig. 5).

Results show the predominance of Pb and Fe, respectively, in the range of 0.32 ± 0.09–0.70 ± 0.28 mg/mL and 0.62 ± 0.3–0.89 ± 0.13 mg/mL followed by Cu and Zn in the range of 0.06 ± 0.007–0.096 ± 0.006 and 0.05 ± 0.02–0.16 ± 0.08 mg/mL.

Thus, based on the mean concentration of heavy metals in toad skin biopsy, Pb 0.48 ± 0.15 mg/Kg, Fe 0.72 ± 0.11 mg/Kg, Cu 0.08 ± 0.01 mg/Kg and Zn 0.11 ± 0.05 mg/Kg, they were ranked as follows: Fe > Pb > Zn > Cu.

Based on the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which declare the provisional tolerable weekly intake (PTWI) for Pb equivalent to 0.005 mg/Kg/day, Fe 0.8 mg/kg/day, Cu 2 mg/Kg/day and Zn 1 mg/kg/day (WHO 2008), excessive values detected for Pb which were 96 times higher than the threshold limits.

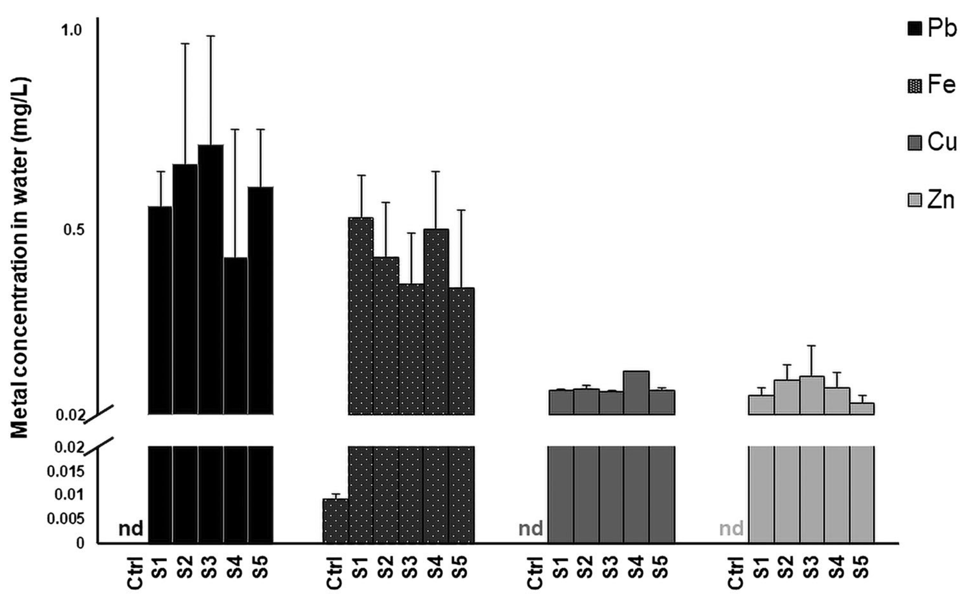

Fig. 3 Metal concentration of Pb, Fe, Cu and Zn (mg/mL) of water samples in sampled sites. nd = metal concentration not detected; Ctrl = control site; S1–S5 = sampled site 1 to 5

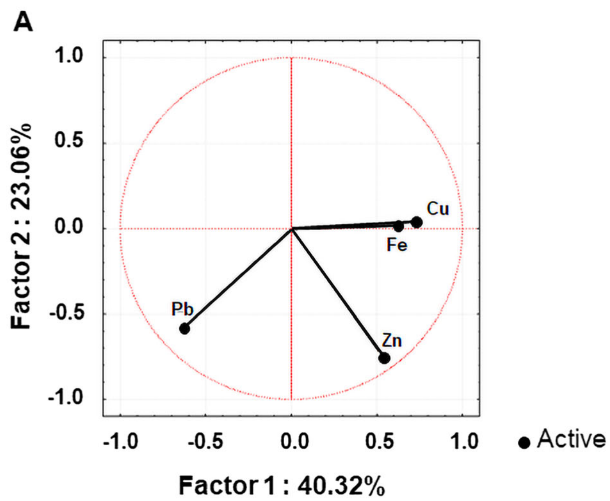

Fig. 4 **a** Principal component analysis of heavy metal concentration in water according to factors, factor-plane (1 × 2); **b** Dendrogram depicting the hierarchical clustering of the heavy metals in water

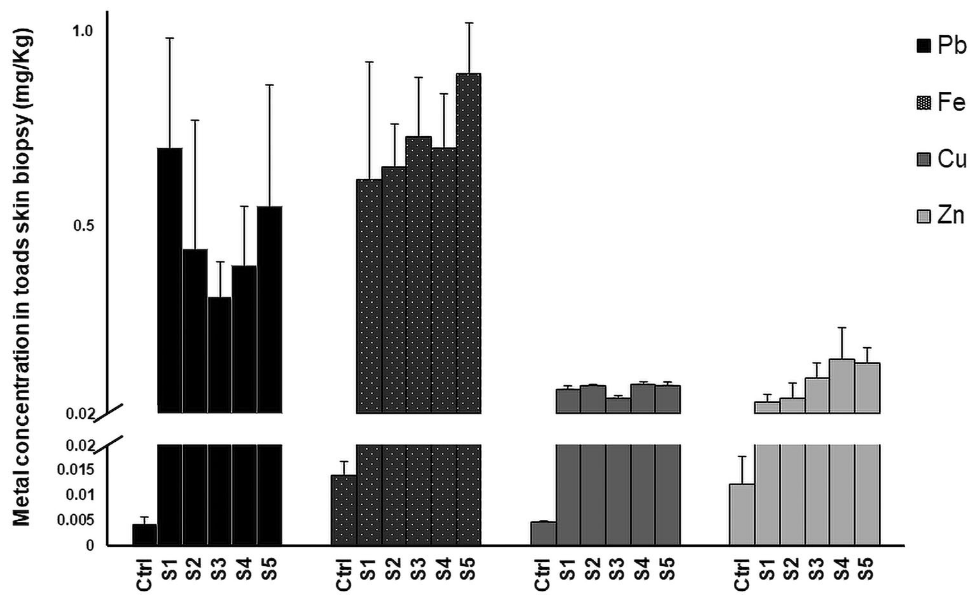

Fig. 5 Metal concentration of Pb, Fe, Cu and Zn (mg/mL) in skin toad *Bufo spinosus* in sampled sites; Ctrl = control site; S1–S5 = sampled site 1 to 5

Discussion

The results presented in this study comprise an attempt to report the heavy metals distributions and their degree of contamination (Pb, Fe, Cu and Zn) in river water and in the skin of the male spiny toad *Bufo spinosus* collected from the Medjerda River basin in the northeastern region of Algeria.

Medjerda river was already explored for natural organic matter (Jouadi et al. 2012) reporting the characterization of the heterogeneous nature and the importance of the various subcomponents characterization. We focus on the heavy metal, the toxic metal Pb and the essential microminerals as Fe, Cu and Zn for human anthropogenic activities detected in the sites examined and their documented effects on aquatic organism in the literature. As kown, all toxic and trace metals interfere in the normal functioning of structural proteins, enzymes, and nucleic acids by binding them. But, recently, in amphibian evidence of metabolic and enzyme activities alterations, morphophysiological changes were reported after increasing levels of Pb, Fe and/ or Cu and Zn alone or combined (Pinelli et al. 2019; Chagas et al. 2020).

In the present study, five different localities based on human anthropogenic activities and presence of toads were chosen. As shown in the results, even though the concentrations of Pb, Fe, Cu and Zn in water samples were evenly distributed among the five sampling stations, they strongly differ from the control area where most of heavy metals were undetectable.

Further by the dendrogram, the concentrations in the water samples of Cu and Zn result homogenous as cluster as well as the other cluster of Fe and Pb. Since copper is susceptible to oxidation, the observed cluster of native copper with zinc in water is of interest and deserves our future attention.

The presence of copper mainly comes into the environment through manufacturing industries such as paint and leaded gasoline, tire wear, lubricating oil, grease, batteries production and ceramics, remains of batteries whereas the concentration of zinc in the water could be due to leaching effects from several sources as refineries, brass manufacture, metal plating, plumbing.

High levels of iron in water sources can been attributed to the geology of the area, the main factor controlling groundwater hydrology whereas the entry of sewage, caused by the activities of agriculture, industry and domestic sewage as automobile emission, mining, burning of coal into these ecosystems, can be the reasons for the high levels of lead in Medjerda River.

In addition, lead concentration was approximately 60 times higher than the permissible limit of the metal in water (WHO 2008). These data indicate that these sites contain point sources of heavy metal pollution from where these pollutants are dispersed into the surrounding area. In fact, the sampling site 1 is located near the spillways of H'Nancha city where, due to a lack of sewage pipelines, the urban and domestic discharges are injected directly into the river. Sampling sites 2 and 3 are situated upstream and downstream of Ain Dalia Dam respectively. It is an area used for washing wool and automobiles as well as where manufacturing of traditional building materials takes place and where visible signs of water pollution such as dark watercolour and a foul smell is observed. Sampling site 4 located close to the Zaarouria village, south of the city of Souk-Ahras, is characterized by a waste treatment system which discharges into the river, while the site 5 is located in a zone of waste water discharges characterized by poultry-raising activity at the edge of the river.

To study the effect of toxic and trace metals examined on biota we chosen amphibian for their key ecological role as bioindicator (Niethammer et al. 1985; Ben Hassine and Escorzia 2017; Guerriero et al. 2018b; Pinelli et al. 2019). In particular, for this project we collected skin biopsy from male toads because anatomically they are very easy to identify and to complete gender detoxification analysis of cytochrome aromatase p450 aromatase and glutathione S-transferase in *Bufo spinosus* by Real Time-PCR (*studies in progress*).

Our present results on the concentration of the toxicants, heavy metals in the skin tissue showed high values compared to the control area, which were found within permissible limits.

These results in the skin tissue showed higher Pb concentration than the permitted threshold established by JECFA. In fact, Pb was the only heavy metal with higher levels relative to threshold limits than of the other heavy metals detected in the same areas. Several previous studies on heavy metals, specifically on Pb, have reported a wide range of biological and neurological effects in different organisms (Assi et al. 2016; Lasley 2018; Rocha and Trujillo 2019), reproductive toxicity (Guerriero 2007; Huang et al. 2017; Rameshbhai et al. 2019), embryotoxicity (Lee and Ko 2017), teratogenicity (Lee and Ko 2017), as well as effects on mutagenicity and carcinogenicity (Thanomsangad et al. 2019).

Toads need moist skin, particularly during the development of embryos and larvae (Unrine et al. 2007; Zhang et al. 2007). Their semipermeable and highly vascularized skin permits cutaneous gas exchange, resulting in toads being highly susceptible to heavy metal accumulation. In fact, heavy metals can be absorbed via their skin and the intestine and reach to target tissues through circulatory system (Stolyar et al. 2008; Burlibaşa and Gavrilă 2011; Simon et al. 2011; Thanomsangad et al. 2020). Furthermore, heavy metal accumulation in toads strongly depends not only on the metal concentrations present in the environment but also on other factors including the affinities of metals to different tissues, different rates of uptake and deposition, the metal distribution in various organs and the excretion rate (Putshaka et al. 2015; Guerriero et al. 2018b).

At the same time, the high levels of heavy metals in tissue as well as in the water can also be explained by the fact that the typical organisms on which toads prey are insect larvae, crickets, dragonflies, earthworms, grasshoppers, and isopods (Burlibaşa and Gavrilă 2011) which in turn may contain high concentrations of heavy metals due to contact with the contaminated environment. In fact, heavy metals in sediment, soil and water may be inadvertently ingested by toads during their prey capture (James and Semlitsch 2011).

Conclusions

Investigating the chemistry of surface water obtained from the Medjerda River basin has given an insight into impact of land use effect on water quality. Analyses of the various ions has indicated that Pb, Fe, Cu and Zn in water samples were found to be evenly distributed among the five sampling stations. These results reveal Pb concentrations approximately 60 times higher in comparison to permissible limits of heavy metals for water quality (WHO 2008).

Thus, our study provided an opportunity to evaluate the spiny toad *Bufo spinosus* as a potential monitor of heavy metal contamination. This species performs as an appropriate biosentinel, accumulating pollutants in levels representative of the environmental contamination degree while still able to survive, being relatively sedentary, ubiquitous in situ and easily collected. The combination of these characteristics with a non-invasive skin removal method for heavy metal detection, allows reduction of the number of samples needed to monitor the levels of metal contaminants as well as enabling identification of problem areas and evaluation of the fate of these contaminants.

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Author Contributions All authors contributed to conception and design of the experiments. All the authors have given their approval to the final version of the manuscript.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interest.

Human and animal rights This study was conducted in strict accordance with European (Directive 2010/63) legislation on the care and use of animals for scientific purpose.

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