water

MDPI

Article

Non-Lethal Assessment of Land Use Change Effects in Water and Soil of Algerian Riparian Areas along the Medjerda River through the Biosentinel *Bufo spinosus* Daudin

Pasquale Napoletano [1,*], Noureddine Guezgouz [2], Imen Benradia [2], Sarra Benredjem [2], Costantino Parisi [3], Giulia Guerriero [3,4,5,6,†] and Anna De Marco [6,7,*,†]

1 Department of Agricultural, Environmental and Food Sciences, University of Molise, 86100 Campobasso, Italy
2 Department of Biology, University of Mohamed Cherif Messaadia, Souk-Ahras 41100, Algeria; n.guezgouz@univ-soukahras.dz (N.G.); i.benradia@univ-soukahras.dz (I.B.); s.benredjem@univ-soukahras.dz (S.B.)
3 Department of Biology, University of Naples Federico II, 80126 Naples, Italy; parisi.eclab@gmail.com (C.P.); giulia.guerriero@unina.it (G.G.)
4 Mediterranean and Middle East Universities Network Agreement (MUNA), University of Naples Federico II, 80126 Naples, Italy
5 Interdepartmental Research Centre for Environment (CIRAm), University of Naples Federico II, 80134 Naples, Italy
6 Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology (BAT Center), University of Naples Federico II, 80055 Portici, Italy
7 Department of Pharmacy, University of Naples Federico II, 80131 Naples, Italy
* Correspondence: pasquale.napoletano@unimol.it (P.N.); ademarco@unina.it (A.D.M.)
† These authors contributed equally to this work.

check for updates

Citation: Napoletano, P.; Guezgouz, N.; Benradia, I.; Benredjem, S.; Parisi, C.; Guerriero, G.; De Marco, A. Non-Lethal Assessment of Land Use Change Effects in Water and Soil of Algerian Riparian Areas along the Medjerda River through the Biosentinel *Bufo spinosus* Daudin. *Water* **2024**, *16*, 538. https://doi.org/10.3390/w16040538

Academic Editors: Patrícia Palma and Alexandra Tomaz

Received: 28 December 2023
Revised: 25 January 2024
Accepted: 7 February 2024
Published: 9 February 2024

Abstract: The land use change can negatively impact ecosystems, enriching water and soil with heavy metals (HMs). The fragile riparian areas along the Medjerda River of Northern Algeria are particularly affected by this phenomenon, and biological tools able to combine information about both matrices can be preferred in their monitoring. This research aimed to evaluate the suitability of the spiny toad (*Bufo spinosus* D.) as a biomonitor for assessing the impact of three different land uses (agricultural (AGR), urban (URB), and industrial (IND) managements) on soil and water for Cu, Fe, Pb, and Zn by using the non-lethal skin biopsy. The IND land use, followed by URB, mostly impacted soils for Cu and Pb, whereas management was not able to influence water differently despite worrying Pb levels. The cluster analysis allows to show that toad responds like soil in terms of land use and like water for HMs, as it is possibly related to the metal–chemical affinity. Although the single skin analyses do not display any difference among the managements, the bioaccumulation factor (*BF* > 1) shows that *B. spinosus* absorbs more HMs from water; skin accumulates Fe and Zn regardless of the land use, probably for both natural and anthropogenic assimilation and Pb and Cu for URB and IND, as it is related to their ionic forms and human impact.

Keywords: agriculture; urban and industrial activities; heavy metal contamination; Medjerda River; skin biopsy; biomonitoring; bioaccumulation factor; amphibian; environmental monitoring

1. Introduction

Biomonitoring has developed notably in the last decades. Generally, biological monitors are organisms able to indicate the level of vitality of living things, at single or community level, and give information about the health status of the environmental compartments that occupy [1]. These organisms experience cumulative effects of several environmental changes in a temporal dimension, in particular, pollutants, estimating past exposure and reflecting individual susceptibility [2]. Bioindicators are, therefore, able to act as both sensitive and accumulative organisms as they undergo morphological and physiological changes caused by contaminants. Given their occupation in different ecological niches, they have the potential to serve as sentinel organisms for assessing the health status of ecological sites. Consequently, the evaluation of ecosystem health can be reflected by the organisms inhabiting it [3]. Different biomonitors are reported in the scientific literature, in detail: earthworms, arthropods, and land snails for soil [4–7]; fish or aquatic macroinvertebrates for water [8,9]; lichens and moss for air [3,10,11]; and also microorganisms and vascular plants (i.e., bacteria, fungi, herbs, and trees) [12–16].

Some biomonitors can indicate environmental changes in more than one compartment, related to the ones in which they spend their own lifecycle, like insects or amphibians. These latter have biphasic lifecycles occurring in both aquatic and terrestrial systems, as they are very sensitive to the consequences of environmental threats, way more than other species due to the peculiarities of their skin [17]. This organ is very complex, responsible for respiration, osmoregulation, thermoregulation, and adsorption, and makes the amphibians valid biosentinels [18]. Previews studies reported the efficacy of *Hyla cinerea* (Garman, 1892), *Ambystoma barbourin* (Kraus and Petranka, 1989), *Rana pipiens* (Schreber, 1782), *Rana clamitans* (Latreille, 1801), *Xenopus laevis* (Daudin, 1802), *Bufotes viridis* (Laurenti, 1768) as rapid responders of environmental contamination and ecological damage [19–21]. Similarly, Guezgouz et al. [22] and Napoletano et al. [23] focused on the efficacy of the anuran *Bufo spinosus* (Daudin, 1803) to act as biosentinel for ecological studies in some Algerian riparian zones. Indeed, this toad is very sensitive to environmental changes as it occupies a wide range of different habitats, including areas with fragmented landscapes. Moreover, it is an Ibero-Maghrebian endemism with a population in Northern Africa (from Morocco to Tunisia) and the Iberian Peninsula, besides the British Isles and Northern Pyrenees, and it is classified as a "least concern" species according to the International Union for the Conservation of Nature (IUCN) [24].

Because of the strict relationship between amphibian health status and the environmental compartments in which they live, their skin is a reflection of changes that occurs in water and soil. The use of innovative techniques, such as non-invasive skin removal after capturing followed by the release of individuals, has proven to be a successful strategy since it minimizes entropic pressure on the amphibian population. This approach is helpful for monitoring overall environmental quality, including soil and water, especially in riparian ecosystems [22,23]. These areas are complex and multidimensional, composed of a gradient of characteristics ranging from terrestrial to aquatic systems [25]. They are hotspots of ecological function in many landscapes and provide several ecosystem services related to the stabilization of river banks, water regulation and supply, flood protection, erosion regulation, storage capacity, nutrient regulation, pollination, aesthetic, amenity, habitat refugium, and biodiversity [26,27]. Human influences are very pronounced in riparian zones, ranging from extreme manipulation to agricultural needs [26]. Here, land use conversion is the major anthropogenic change that reshapes these environments, resulting in considerable risk to both soil and water.

Deforestation, agricultural intensification, and urbanization are the main land uses that led to the modification and fragmentation of riparian areas, degrading soil and water, and loss of biodiversity [28,29]. In developing countries, it has been observed that the conversion of land use and land cover into agricultural lands and urban areas has resulted in various environmental consequences, such as loss of ecosystem integrity, ecosystem imbalance, and a below-optimum provision of ecosystem services [30]. An example of these changes is seen in Africa, mainly in the Mediterranean countries. The expansion of agriculture by local communities to satisfy their needs due to human population growth is an important driver of land use change. In addition, water bodies and different land covers have been reduced by agriculture and urbanization, causing severe contamination by heavy metals (HMs) in these ecosystems. HMs pose intense environmental damage because of their intrinsic toxicity that reflects on persistence, non-biodegradability and bio-accumulation characteristics [31]. Moreover, they can accumulate and remain in water and soil for a long time, also causing diseases and complications for humans [31,32]. The release of HMs is usually dependent on the anthropogenic activity that alters the natural biogeochemical cycles of these elements, emphasizing the effects of land use change. Some HMs, including copper (Cu), iron (Fe) and zinc (Zn), are crucial for metabolism at low concentrations and considered micronutrients for biological activities. However, at high concentrations, they induce toxic effects [33]. Moreover, certain heavy metals, in particular lead (Pb), are described as the most problematic HMs, ranking among the major public health concern chemicals, being able to damage the function of various organs even at low concentrations [22,33].

Pb, alone and together with Cu, resulted as a driver in decreasing biological activity, reflecting high microbial stress and low microbial abundance for the soils investigated by Santorufo et al. [34]. In the comparison of different land uses, these authors showed a marked overall reduction, especially in the agricultural soils, that affected the ecosystem functioning, as compared to forest and urban areas.

However, the effect of land use assessed on the anthropization gradient from forest, grassland, and cultivated in urban, industrial, mining, and military areas is usually related to the increase in Cd, Cu, Pb, and Zn contaminations [35]. In fact, the addition of pesticides and fertilizers to soils [36] and atmospheric fallout from traffic induce metal contamination [37] and can contribute to the gradual increase in HMs from forest land use to more anthropized land uses.

Therefore, there is a growing concern regarding the effects of land use change in sensitive ecosystems such as riparian ones and in biomonitoring methods that use an integrated approach alongside the study of water and soil alone for developing strategies for sustainable management of these areas. In this perspective, the main goal of the current research was to evaluate the suitability of *Bufo spinosus* as a biomonitor for assessing the impact of three different land uses (agricultural, urban, and industrial managements) on soil and water HM concentration in riparian zones of Northern Africa. The research focused on the simultaneous assessment of Cu, Fe, Pb and Zn in soil, water and toad skin from areas with similar edaphic and climatic characteristics but different land use. This aspect was particularly interesting and provided information on the impact of land use at the ecosystem level through a direct comparison that is not limited to one environmental compartment. This integrated assessment of water, soil and organisms could be particularly important in riparian areas. Another focus of this study was to highlight the reactivity of the toad to changes in water and soil HM concentration, and the affinity to one or both environmental compartments by using a non-lethal technique such as skin biopsy. In order to estimate HM accumulation in the toad tissue and describe a preferential transfer from soil or water, the bioaccumulation factor (*BF*) was calculated.

In addition, in agricultural, urban, and industrial areas, the determination of HM concentrations in soil, water and toad skin was accompanied by the calculation of some geochemical and ecological indices that are often considered informative on the degree of contamination, anthropogenic enrichment, and environmental risk even in the absence of an undisturbed control area as in this study [23,38].

2. Materials and Methods

2.1. Study Area and Experimental Design

The investigated riparian areas are located along the Medjerda River in the Souk-Ahras province of Northern Algeria. This river is trans-frontal with headwaters in the Montagnes region before traversing the urban section [22]. It is near the border with Tunisia and has hydrological networks in Souk-Ahras. Its basin extends into Tunisia, and the overall captivity area is about 23,700 km^2. In addition, about 32% (about 7800 km^2) of this land mass lies in Algeria [39]. These territories have been intensively modified by anthropogenic activity, and land uses are diverse and widely distributed. They have a semi-arid climate, featuring hot, dry summers and mild winters, and are often affected by drought as a direct result of climate change that reduced precipitation and increased temperatures [39,40].

The study area comprises agricultural (AGR), urban (URB), and industrial (IND) sites located 20–30 m from the river and only occasionally affected by flooding events over the last years (Figure 1).

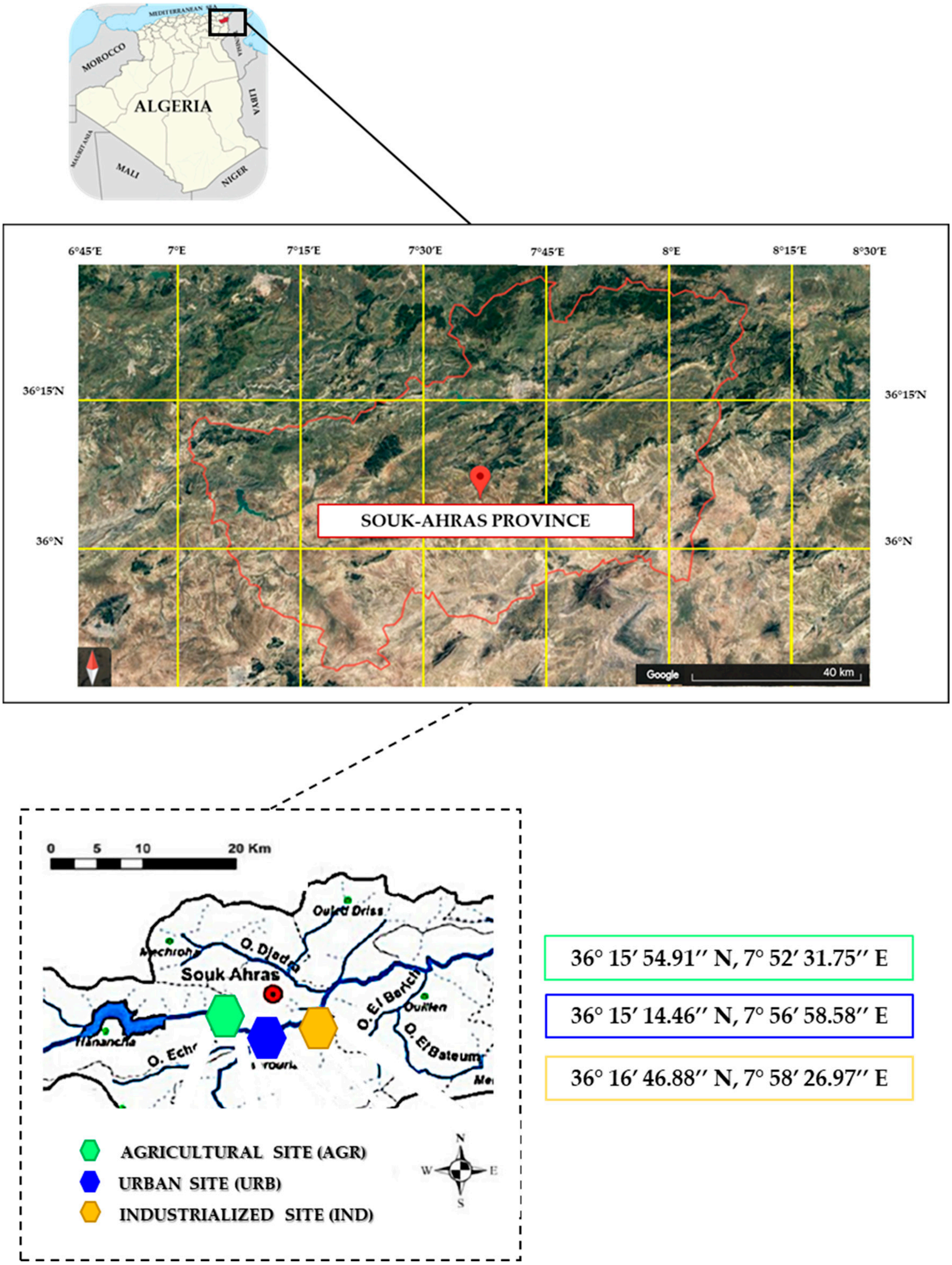

Figure 1. The location of the investigated areas along the Medjerda River in the Souk-Ahras province (Northern Algeria).

All the sites were similar for parent material, pedogenesis, and climatic conditions and, therefore, were chosen for this research. Moreover, each site was about 500 m^2 wide.

Stagnic Fluvisols (AC profiles) were present as soils characterized by deposits of calcareous silt and clay coming from recent alluvions [40,41]. They are weakly developed soils, showing obvious sedimentary stratification for their formation in an alluvial plain during flooding events, and the organic matter distribution is irregular. The soils were covered by horticultural crops, mainly *Cicer arientinum* L., *Hordeum vulgare* L., *Raphanus raphanistrum* subsp. *sativus* L., *Solanum tuberosum* L., *Triticum aestivum* L., and *Vicia lens* (L.) Coss. & Germ. in AGR, whereas perennial and annual herbs, belonging mostly to Amaranthaceae, Apiaceae, Asteracee, Cyperaceae, Malvaceae, Poaceae, Solaneaceae and Zygophyllaceae families in URB and IND sites. In detail, for URB and IND, vegetation was not investigated more.

In this study, the topsoils of 0–10 cm depth (six per site in order to obtain representative samples) were picked up considering that the soil's upper layer generally exerts influences on biota. A sieve of <2 mm was used to remove the soil's bigger particles (aggregates and stones), and then the samples were dried and analyzed for the main pedological characteristics and heavy metals.

Water was collected from the river close to AGR, URB, and IND sites in six points per land use, and for each point, six samples were collected and then mixed to have composite samples. It was then passed through a filter of 0.45 µm in cellulose and kept in a refrigerator at 20 °C in the dark until further investigations.

All the reagents used in this study were provided by Sigma-Aldrich (Milan, Italy) and had the highest purity level (99.98%).

2.2. Soil Physico-Chemical Characterization

The following general parameters were assessed in soil: texture (sand, silt, and clay); water-holding capacity (WHC); pH; organic matter (OM); and total content of heavy metals (Cu, Fe, Pb, and Zn). Following the methods reported in the Italian official methods of soil analysis [42], the particle size (%) was determined gravimetrically by the pipette and wet sieving method after a pre-treatment with 3 mL of hydrogen peroxide (H_2O_2) at 30% for oxidizing organic materials and 10 mL of sodium orthophosphate (Na_3PO_4) at 4% for suspending the particles. WHC (%) was measured by inducing soil saturation with distilled water and, after drying, calculated as ((wet mass − dry mass)/dry mass) × 100. Soil pH was measured potentiometrically in soil as follows: distilled water suspension (1:2.5), whereas OM was evaluated from soil organic carbon (TOC) according to the Walkley–Black wet combustion technique, with $K_2Cr_2O_7$ and H_2SO_4 for 30 min followed by the titration with diphenylamine [43]. Then, TOC was divided by 0.58 to obtain OM content (% d.w.) [44]. The total and available concentration of Cu, Fe, Pb, and Zn (values in ppm) was determined by atomic absorption spectrophotometry (VARIAN model SpectrAA-20, Varian Associates Inc., Sunnyvale, CA, USA). The calibration curves for the instrument were obtained after diluting pure standards (1000 ppm) in acidic solutions, similar to the digested soil samples. Accuracy was checked by concurrent analysis of standard reference material (BCR CRM 142R—Commission of the European Communities, 1994, Joint Research Centre Institute for Reference Materials and Measurements, Geel, Belgium). The overall element recovery ranged from 80 to 120% for all the investigated samples. In detail, to determine the HM total concentration, 2 g of soil was digested with 12 mL of HNO_3 at 65% and 6 mL of HF at 50% (1:2 = *v*:*v*). Soil samples were oven-dried (105 °C) and grounded into a fine powder by an agate mortar (Fritsch Analysette Spartan 3 Pulverisette 0, Fritsch, Idar-Oberstein, Germany) before acid digestion in a microwave oven (Milestone-Digestion/Drying Module mls 1200, MLS GmbH Mikrowellen-Laborsysteme, Leutkirch, Germany). The available fractions of Cu, Fe, Pb, and Zn were determined in extracted soils according to Lindsay and Norwell [45]. Briefly, 25 g of oven-dried (105 °C) soil samples were added to 50 mL of diethylenetriamine pentacetic acid (DTPA), $CaCl_2$ and triethanolamine (TEA) solution at pH 7.3 ± 0.05. The soil suspensions were shaken for 2 h and filtered with the Whatman

No. 42 filters. The available fractions of Cu, Fe, Pb and Zn are reported both as concentration values in ppm and as a percentage of the total content of each HM.

The total content of each HM was used for the calculation of soil indices in order to display differences in land uses starting from geochemical background and reported in other works [38,46]. The following indices were evaluated: geo-accumulation index (*Igeo*); enrichment factor (*EF*); contamination factor (*CF*); and potential ecological risk index (*RI*).

Igeo indicates the specific HM contamination in the specific soil and land use by comparing the current concentration with the natural level used as a reference and calculated as reported in Equation (1) [23,47].

$$Igeo = \frac{\log_2[sample]}{1.5 \times [reference]} \tag{1}$$

where [*sample*] is the concentration of HM in the soil and [*reference*] is the geochemical background of the same metal [48]. A factor of 1.5 is applied to minimize value fluctuations due to the wide heterogeneity of the Earth's crust. Therefore, *Igeo* can be grouped as uncontaminated (*Igeo* 0), uncontaminated to moderately contaminated (*Igeo* 0–1), moderately contaminated (*Igeo* 1–2), moderately to strongly contaminated (*Igeo* 2–3), strongly contaminated (*Igeo* 3–4), strongly to extremely strongly contaminated (*Igeo* 4–5), and extremely contaminated (*Igeo* > 5).

EF estimates the amount of a given HM derived from human activities by comparing the specific metal in the sample with an uncontaminated background after normalization with Fe [49]. The formula is reported in the Equation (2):

$$EF = \frac{\frac{[element]}{[normalizer]}\text{sample}}{\frac{[element]}{[normalizer]}\text{reference}} \tag{2}$$

where [*element*] and [*normalizer*] are the concentration of the investigated metal and Fe in the sample and background, respectively. The classes are as follows: *EF* < 2 deficiency to minimal enrichment; *EF* = 2–5 moderate enrichment; *EF* = 5–20 significant enrichment; *EF* = 20–40 very high enrichment; *EF* > 40 extremely high enrichment.

CF describes the degree of the contamination in HMs related to background levels as reported in the following Equation (3) [50,51]:

$$CF = \frac{Cn}{Bn} \tag{3}$$

where *Cn* is the content of HM and *Bn* is the same metal concentration in the average composition of the continental crust. *CF* < 1 means low contamination; $1 \leq CF < 3$ means moderate contamination; $3 \leq CF < 6$ means considerable contamination, and $CF \geq 6$ means very high contamination.

RI provides information on the impact of HMs on environmental and ecological processes according to the Equation (4) [52]:

$$RI = \sum E_r^i \tag{4}$$

where E_r^i represents the ecological risk factor of the HM calculated from *CF* and the toxic response of the specific HM *i* (T_r^i) [23]. This index follows the following classes: low risk (*RI* < 150); moderate risk ($150 \leq RI < 300$); considerable risk ($300 \leq RI < 600$); and very high risk ($RI \geq 600$).

2.3. Water Evaluation

During sampling at each site, the main chemical characteristics such as pH, dissolved oxygen (DO) and biological oxygen demand (BOD) were measured in situ using a portable multi-parameter apparatus WTW multiline P4 (WTW GmbH, Weilheim, Germany). The turbidity was determined through a nephelometric turbidimeter (2100N, Hach, Loveland, CO, USA). The content of heavy metals was assessed after the addition of HNO_3 at 3% (*v:v*) using an atomic absorption spectrophotometer (Shimadzu AA 6200, Shimadzu, Japan) [22].

2.4. Toad Collection and Skin Analysis

Adult spiny toads were identified as *Bufo spinosus* (Daudin, 1803) after being randomly captured with a standard technique by pitfall traps [53]. Only male individuals, in accordance with their phenotypic and behavioral traits, ranging from 180 to 200 g, were chosen. The collected toads were, in total, six per area, sampled for water and soil (six field replicates for each land use). The toads were subjected to an in situ skin biopsy by scalpel after ether somministration. This method was based on a non-lethal protocol characterized by the collection of 1 cm^2 of skin from each specimen [54]. Then, the animals were released after a short period of recovery from the stress (ca. 20 min). The pool of collected skin was dried at 120 °C for 2 h in a ventilated oven, and aliquots of 500 mg were treated with HNO_3 at 69%. After that, the digestion was facilitated by leaving the aliquots for five days at about 25 °C; thus, a suspended grease was obtained. These samples were filtered twice and diluted with Milli-Q ultrapure water (Millipore, Milan, Italy) in the tubes to a final volume of 10 mL. A VARIAN SpectrAA-20 was used for metal determination as reported for soil investigations.

2.5. HM Bioaccumulation

The bioaccumulation factor (*BF*) was calculated in order to estimate HM accumulation in the toad tissue, describing the transfer of these elements from water and soil (Equation (5)):

$$BF = \frac{Ct}{Ce} \tag{5}$$

where *Ct* is the metal content in the toad's skin, and *Ce* is the available content of the same element in the specific environmental compartment, soil (*BFs*), or water (*BFw*) [55]. Three classes of bioaccumulation were possible: $BF > 1$ means that the toad skin can be regarded as an accumulator; $BF < 1$ means that it acts like an excluder, and $BF = 1$ means that there is no influence [56].

2.6. Statistical Approach

The results of soil, water characteristics, and toad metals were reported as means ± standard errors. Specifically, soil and water were means of 6 field points per site × 3 laboratory replicates, while toad metals were means of 6 specimens per site × 3 laboratory replicates. The Shapiro–Wilk test was used to assess if the data had a normal distribution, and One-Way Analysis of Variance (ANOVA) was tested for the significance of differences ($p \leq 0.05$).

Hierarchical clustering was performed for soil, water, toad, and the merged dataset of soil + water + toad in order to highlight differences and similarities among land uses and metals. The paired group (UPGMA) with the Bray–Curtis similarity index was selected.

Systat_SigmaPlotsoftware version 12.2 (Jandel Scientific, San Rafael, CA, USA) and Past version 4.03 (Øyvind Hammer, Oslo, Norway) were used in this research.

3. Results

3.1. Soil and General Water Characteristics

The general characteristics of the investigated soils and water collected in three different areas subjected to different land uses, i.e., agricultural (AGR), urban (URB), and industrialized (IND) in riparian zones of Northern Africa, are shown in Tables 1 and 2, respectively.

Table 1. General soil characteristics (particle size %: sand, silt, and clay; water-holding capacity (WHC); pH; and organic matter (OM) % d.w.) evaluated in the sites subjected to different land use (AGR, URB, and IND) and reported as mean ± standard error. Different letters show statistically significant differences among different land use (One-Way ANOVA, $p \leq 0.05$).

	Particle Size (%)					
	Sand	Silt	Clay	WHC (%)	pH	OM (% d.w.)
AGR	66.8 ± 1.8	21.4 ± 1.6 ab	11.8 ± 1.2	30.7 ± 1.5	8.49 ± 0.05 b	9.21 ± 0.84 a
URB	65.1 ± 1.6	23.5 ± 1.5 a	11.4 ± 1.4	29.8 ± 0.9	8.66 ± 0.05 a	7.15 ± 0.22 b
IND	67.0 ± 1.7	20.6 ± 1.2 b	12.4 ± 1.6	30.1 ± 1.8	8.31 ± 0.10 b	6.92 ± 0.82 b

Table 2. General water characteristics (pH, turbidity, dissolved oxygen (DO), and biochemical oxygen demand (BOD)) evaluated in the sites subjected to different land use and reported as mean ± standard error. Different letters show statistically significant differences (One-Way ANOVA, $p \leq 0.05$).

	pH	Turbidity (NTU)	DO (mg L^{-1})	BOD (mg L^{-1})
AGR	6.4 ± 0.4	4.0 ± 0.3	9.8 ± 0.3 a	6.9 ± 0.2 a
URB	6.3 ± 0.5	3.7 ± 0.3	9.1 ± 0.2 b	6.3 ± 0.3 b
IND	6.0 ± 0.2	3.5 ± 0.5	9.0 ± 0.4 b	6.2 ± 0.2 b

Regardless of the land uses, the soils (Table 1) were generally sandy (>60% particles in weight) with more than 11% in clay, having alkaline pH (>8), WHC around 30%, and high content of OM (>6.9% d.w.). They resulted in statistical differences in silt, pH and OM. In detail, URB showed the highest content of silt (23.5 ± 1.5%) and pH (8.66 ± 0.05). In addition, AGR soil showed the highest values of OM (9.21 ± 0.84% d.w.); IND soil showed the lowest value (6.92 ± 0.82% d.w.) but was statistically similar to URB (7.15 ± 0.22% d.w.).

On the other hand, water (Table 2) did not evidence any statistical difference in terms of pH and turbidity, showing a trend as AGR > URB > IND. Conversely, DO and BOD showed higher values (9.8 ± 0.3 mg L^{-1} and 6.9 ± 0.2 mg L^{-1}, respectively) in water near AGR sites than URB (9.1 ± 0.2 mg L^{-1} and 6.3 ± 0.3 mg L^{-1}) and IND sites (9.0 ± 0.4 mg L^{-1} and 6.2 ± 0.2 mg L^{-1}).

3.2. HM Concentration in Water, Soil and Toad Skin

The available fractions of Cu, Fe, Pb, and Zn for soil and the content of these elements in water and toad skin were reported in Figure 2. In addition, the soil total content of HMs is shown in Supplementary Figure S1. The studied land uses only statistically impacted soil metals, regardless of water or toad. In detail, IND was the most contaminated soil, reflecting the highest total and available concentrations of metals.

Cu and Fe availability was significantly higher in IND soil (Cu: 3.97 ± 0.12 ppm; Fe: 10.82 ± 0.32 ppm) than in AGR and URB soils (Figure 2A). Pb was significantly more available in IND and URB soils (1.89 ± 0.06 ppm and 1.93 ± 0.04 ppm, respectively), while Zn was higher in IND and AGR soils (2.83 ± 0.24 ppm and 2.17 ± 0.24 ppm, respectively) (Figure 2A). This trend is respected by the total metal content in soil under different land use (Supplementary Figure S1).

The general trend of heavy metal availability in soils was Fe > Cu > Zn > Pb, while for water and toads it was Pb > Fe > Cu > Zn and Fe > Pb > Zn > Cu, respectively.

Metal concentrations in water and toad skin (Figure 2B,C) showed greater variability than in soil (Figure 2A), and there was no statistically significant difference among sites with different land uses.

Analyzing the percentage of available metal in the soil over the total quantity, it is evident that Pb > Cu > Zn > Fe (Table 3). The percentage of available Pb and Cu was significantly high in AGR soil, with values of 70.5% and 54.4%, respectively. The availability of Fe was 0.16% in IND soil and 0.14% and 0.12% in AGR and URB soil, respectively (Table 3). No statistical difference was found for the available percentage of Zn, which ranged from 11.7% in URB soil to 15.3% in IND soil (Table 3).

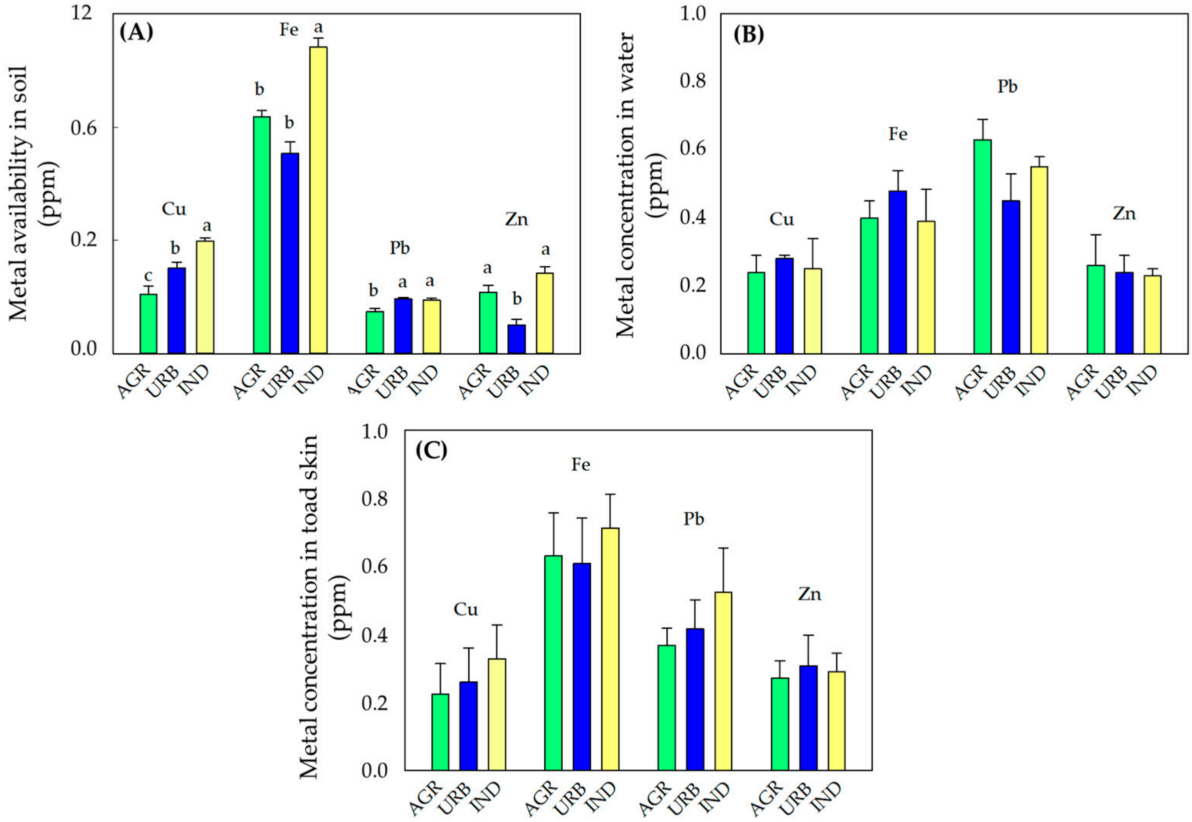

Figure 2. Metal concentration (ppm) of Cu, Fe, Pb, and Zn reported as mean ± standard error in soil (**A**), water (**B**), and toad skin (**C**) subjected to different land uses. For soil, the available fraction of metals is shown. Different letters show statistically significant differences (One-Way ANOVA, $p \leq 0.05$).

Table 3. Available fraction of Cu, Fe, Pb, and Zn expressed as % of total content ± standard error evaluated in the soils subjected to different land use (AGR, URB, and IND). Different letters show statistically significant differences in different land uses (One-Way ANOVA, $p \leq 0.05$).

	Cu	Fe	Pb	Zn
		%		
AGR	54.4 ± 3.0 a	0.14 ± 0.01 ab	70.5 ±2.2 a	13.6 ± 2.0
URB	52.1 ± 2.2 a	0.12 ± 0.01 b	65.5 ± 0.5 b	11.7 ± 2.4
IND	37.4 ± 3.0 b	0.16 ± 0.01 a	64.7 ± 0.8 b	15.3 ± 2.1

3.3. HM Contamination Indices

The potential ecological risk index (*RI*) showed the strongest impact of metals on the URB area (considerable risk), followed by IND and AGR areas with moderate risk (Table 4). In terms of the geo-accumulation index (*Igeo*), all the soils were uncontaminated regardless of the studied HMs. For the enrichment factor-*EF*, IND showed moderate enrichment for Cu, very high enrichment for Zn, and either deficiency or minimal enrichment for Pb (Table 4). However, *EF* showed significant enrichment for Zn in all sites (values between 10 and 20). Similarly, the contamination factor (*CF*) suggested moderate and considerable contamination for Zn in the URB, AGR, and IND areas, respectively (Table 4).

Table 4. Environmental indices calculated to evaluate the extent of contamination or enrichment of a specific element in soils under different land uses (AGR, URB, and IND). The geo-accumulation index (*Igeo*), enrichment factor (*EF*), contamination factor (*CF*), and potential ecological risk index (RI) are reported as mean ± standard error. In brackets, the range for classification of heavy metal contamination is shown. Different letters show statistically significant differences among different land use (One-Way ANOVA, $p \leq 0.05$).

		Cu			Fe		Pb			Zn		
	RI (≤150–≥600)	*Igeo* (0–≥5)	*EF* (≤2–≥40)	*CF* (≤1–≥6)	*Igeo* (0–≥5)	*CF* (≤1–≥6)	*Igeo* (0–≥5)	*EF* (≤2–≥40)	*CF* (≤1–≥6)	*Igeo* (0–≥5)	*EF* (≤2–≥40)	*CF* (≤1–≥6)
AGR	316 ± 60 b	0.06 ± 0.009 b	1.39 ± 0.23 c	0.28 ± 0.05 c	0.04 ± 0.002 ab	0.20 ± 0.007 ab	0.025 ± 0.003 b	0.63 ± 0.07 b	0.13 ± 0.01 b	0.67 ± 0.12 ab	17.06 ± 3.41	3.32 ± 0.59 a
URB	537 ± 25 a	0.08 ± 0.005 b	2.14 ± 0.13 b	0.40 ± 0.02 b	0.04 ± 0.003 b	0.19 ± 0.01 b	0.03 ± 0.001 a	0.94 ± 0.07 a	0.17 ± 0.003 a	0.45 ± 0.13 b	11.28 ± 2.85	2.22 ± 0.65 b
IND	390 ± 58 b	0.16 ± 0.02 a	3.46 ± 0.41 a	0.79 ± 0.09 a	0.05 ± 0.003 a	0.23 ± 0.02 a	0.03 ± 0.001 a	0.77 ± 0.06 ab	0.17 ± 0.004 a	0.80 ± 0.10 a	18.06 ± 3.0	3.99 ± 0.50 a

3.4. HM Bioaccumulation in Toad Skin

The bioaccumulation factor was calculated to estimate HM accumulation behavior and to describe the transfer of these elements from water and soil to the toad skin, the values of which are reported in Table 5 and Supplementary Table S1. Indeed, it showed values <1 for soil and toad skin (*BFs* are in Table 5 and Supplementary Table S1). *BFw* from water to toad skin showed values above 1 for Fe and Zn regardless of land use. Cu and Pb showed bioaccumulation (*BFw* > 1) only in the IND and URB sites, respectively (Table 5 and Supplementary Table S1).

Table 5. The bioaccumulation factors (*BF*) of heavy metals (Cu, Fe, Pb, and Zn) from water (*BFw*) and soil (*BFs*) to toad for sites subjected to different land use (AGR, URB, and IND). Dark grey cells indicate values > 1; light grey cells indicate values < 1.

	BFw				*BFs*			
	Cu	**Fe**	**Pb**	**Zn**	**Cu**	**Fe**	**Pb**	**Zn**
AGR	[light grey]	[dark grey]	[light grey]	[dark grey]	[light grey]	[light grey]	[light grey]	[light grey]
	Fe > Zn > Cu > Pb				Pb > Zn > Cu > Fe			
URB	[light grey]	[dark grey]	[dark grey]	[dark grey]	[light grey]	[light grey]	[light grey]	[light grey]
	Fe = Zn > Pb > Cu				Zn > Pb > Cu > Fe			
IND	[dark grey]	[dark grey]	[light grey]	[dark grey]	[light grey]	[light grey]	[light grey]	[light grey]
	Fe > Zn > Cu > Pb				Pb > Zn > Fe > Cu			

Based on the bioaccumulation factor, the order in which metals were bioaccumulated in toad skin from water and soil varied: in detail, the order for *BFw* was Fe > Zn > Cu > Pb in AGR and IND sites and Fe = Zn > Pb > Cu in URB site (Table 5). In addition, the order for *BFs* was Pb > Zn > Cu > Fe in AGR, Zn > Pb > Cu > Fe in the URB site, and Pb > Zn > Fe > Cu in the IND site (Table 5).

3.5. Hierarchical Clustering

The hierarchical clustering that showed similarities among the land uses and metal behavior was displayed in Figures 3 and 4, respectively.

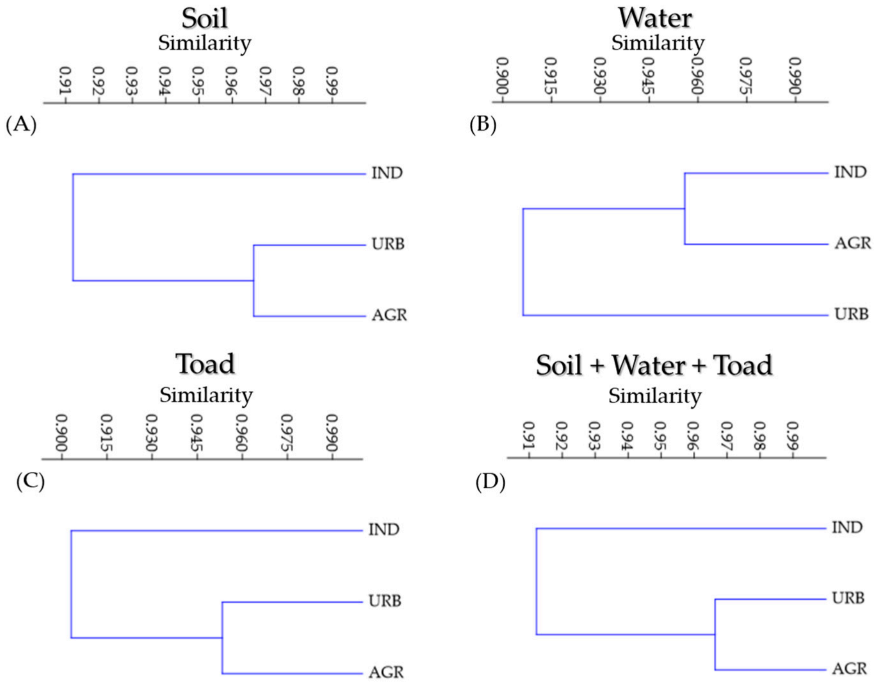

Figure 3. Hierarchical clustering displaying similarity among land uses (AGR—Agricultural site; URB—Urban site, and IND—Industrial site) for soil (**A**), water (**B**), toad (**C**), and soil + water + toad (**D**). The paired group (UPGMA) with the Bray–Curtis similarity index was used.

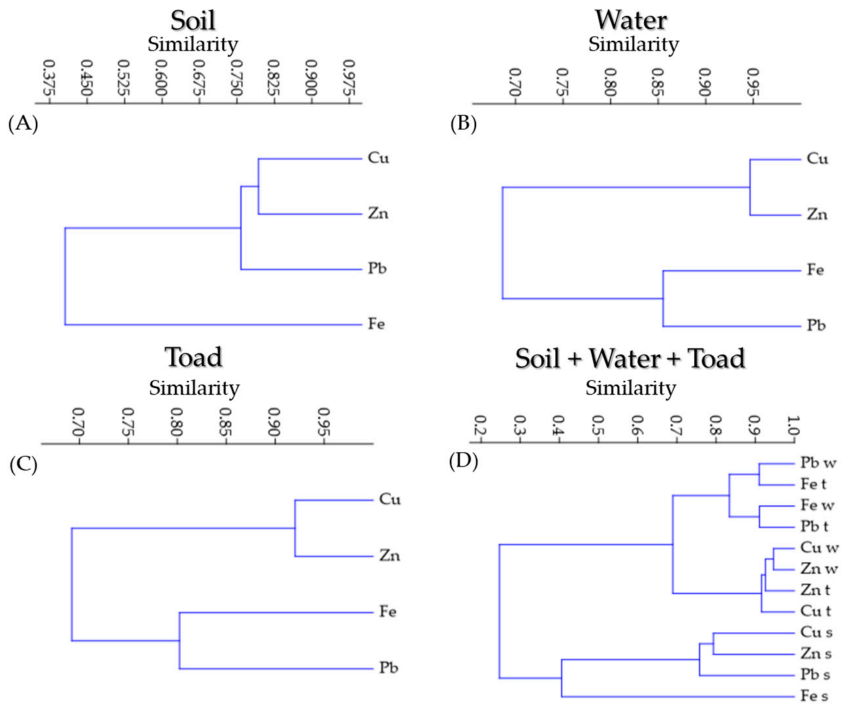

Figure 4. Hierarchical clustering displaying similarity among heavy metals for soil (**A**), water (**B**), toad (**C**), and soil + water + toad (**D**). The paired group (UPGMA) with the Bray–Curtis similarity index was used.

Focusing on the datasets of soil, toad, and the merged matrix of soil + water + toad, the closeness between URB and AGR is shown in Figure 3A,C,D. The distinctive land use was the industrial activity. Water alone highlighted a similarity between IND and AGR and increased dissimilarity for urban sites (Figure 3B).

On the other hand, the dissimilarity index for metals evidenced a similar behavior of Cu and Zn and Fe and Pb in water and toads (Figure 4B,C). Conversely, in soil, Fe differed from other metals (Figure 4A).

The dataset soil + water + toad showed three groups (Figure 4D). The first one was represented by the soil metals, in which Fe constituted the first subgroup with the oldest differentiation and Pb as the second subgroup. The second group was represented by the closeness between Pb and Fe, and the third one by Cu and Zn with alternating positions between water and toads.

4. Discussion

4.1. Impact of Land Uses on Soil and Water

This research focuses on the investigation of different land uses on soil and water in riparian areas and proposes the use of toads to assess the impact on organisms living in these areas. The evaluations were carried out in detail along the Medjerda River in the Souk-Ahras province (Northern Algeria). Our findings highlight that the investigated soil and water respond differently to IND, URB, and AGR land uses in terms of management.

These two compartments have different intrinsic properties as the soil has historical memory, displaying the result of different dynamics occurring in it, compared to water, which is a more dynamic system and, therefore, is less conservative [57,58]. Despite different characteristics, soil and water interact, influence each other, and drive the lives of the organisms inhabiting them.

For the soil compartment, the land use IND induces the enrichment in HMs as it is possibly related to the intense industrial activities that discharge toxic particulate matter in the surrounding zones, mainly containing Zn, Cu, and Pb [59]. In this view, Guasmi et al. [60] have reported that the entire study area has undergone regional deterioration over the last decade, mainly because of industries of paper production, paintings, brick, and textile factories. For these reasons, these anthropogenic activities are the main drivers for high enrichment and contamination indices, as also reported by Napoletano et al. [23]. Particularly interesting is also the comparison with the results of Ben Ayed et al. [61], who reported particularly high contamination with Pb and Cd in five industrial areas along the Medjerda River. In addition, URB was the second most polluting land use due to it being affected by vehicular emissions and open burnings that released contaminants, especially Pb [62]. Santorufo et al. [34] also evidenced a high concentration of lead in the soils of urban areas, addressing the same explanation. This enrichment led to the classification of these soils as considerably risky according to the *RI* index, with an intense impact on ecological processes. Moreover, although Zn is commonly reported to cause pollution, and the values for the enrichment and contamination factors are high, its concentration in the investigated soils cannot be considered high or impactful as it falls within the range of 10–300 ppm for unfertilized and uncontaminated soils, as reported by Noulas et al. [63]. Similarly, AGR land use reflects high but not critical values of Zn in its soils compared to the other managements, and it seems to have more lithogenic than anthropogenic derivation [64]. Indeed, we cannot exclude a partial contribution of these elements (e.g., Zn and Fe) from the parent material as background level meant the soil's memory. However, high levels of Zn are reported along the Medjerda River in Tunisian territory due to specific mining activities, even though the interruption of activities had severely reduced inputs of this element [65]. Anyway, none of the investigated HMs exceeded the regulatory limits adopted in Algeria from the Agence Française de Normalization (AFNOR) [66]. Also, the different leachability of these HMs from the soil, along with the different anthropic contributions, may be the key drivers for water contamination [22]. The dynamic nature of water that flows and dilutes could be the possible reason for the marked differences not being reported among the studied HMs for AGR, URB, or IND land uses [67]. However, the only alarming HM is Pb, which exceeds the established threshold of 0.01 ppm for all sites, according to the World Health Organization (WHO) [68] and reported for waters of nearby areas [22].

The investigated land uses, usually reported to have a direct impact on these environmental compartments in terms of HMs, as also reported by Joimel et al. [35] for their case study in France, also drive general soil and water characteristics. In these terms, the evidence is shown in URB soils where the alkaline pH is the result of both pedological carbonates or external contributions related to requalification policies commonly carried out for urban greening [69,70]. All the investigated land uses show high values of OM (>4%), but AGR highlighted the highest values as agricultural soils need to cope with the continuous production and removal of green biomasses and, therefore, have to be supported by different fertilization plans over time, linked to the specific planted herbs [71,72]. Regardless of the land use, all the investigated soils show sandy texture, with slight differences in silt contents dependent on soil spatial variability. However, OM and texture do not seem to be directly responsible for WHC in soils of any of the studied land uses [23]. On the other hand, AGR, IND, and URB land uses significantly impact water for DO and BOD. These characteristics are generally used as proxies for water quality as DO considers the dissolved oxygen that is essential for the aquatic biota, and BOD defines the quantity of organic matter available for oxygen-consuming bacteria [73]. They generally reflect a similar trend and can be influenced by different properties of the catchment, soil typology and land use [74]. Moreover, the intense agricultural production supported by the uncontrolled use of fertilizers and other long-term malpractices are probably the possible causes of higher values in AGR water [75].

4.2. Responses to Different Land Uses of Bufo spinosus and Relationships with Soil and Water Matrices

The use of amphibians for monitoring environmental quality is a common practice in riparian ecosystems [19,20], as influenced by processes occurring in soil and water. Their biphasic lifecycle makes these biomonitors to be effectively engaged as biosentinels. On the other hand, the non-lethal technique of skin biopsy is quite rare despite this method permitting the collection of skin samples without killing the animals through an environmentally friendly approach. The analysis of such material did not display any difference in the investigated land uses even if several factors, such as the affinity of HMs, deposition, rates of uptake, and excretion, cannot be excluded [76,77]. Generally, the uptake of HMs depends on the skin thickness, as a decreased amount of epidermal cell layers implies a decreased passage length for diffusion [78]. In addition, further morphological characteristics, such as the amount of glands, may influence dermal absorption. In this context, the chemical form of HMs is important as they are potentially absorbed by living organisms as ions [79].

In terms of relationships between toads, soil, and water for land uses, the industrial activities (IND) seem to be the most impactful for soil and toads, whereas URB impacts more water when only considering the management for each investigated matrix. The merged dataset soil + water + toad confirms the strongest environmental impact of the IND management, which is probably related to the combined heavy-metal contribution of each matrix. Some important information is then obtained from the behavior of heavy metals in individual matrices. Indeed, water and toads reflect the same behavior compared to soil. This trend could probably be due to the lithological origin of the studied elements that drive the difference for soil. When we analyze the matrices together, the chemical affinity of the HMs in water and toads is shown. Hence, the major difference in HMs for soil allows them to cluster first. This different grouping is possibly associated with the similarity in chemical forms, more soluble like ions for water and toads compared to the soil's more stable compounds like sulfates and phosphates, despite the available fraction being taken into account [79]. However, the different percentages of the available fraction of HMs allow us to show that Fe and Zn in the soil probably come from parent rock material while Cu and Pb are of anthropic contribution. In addition, for these latter elements, their high percentage available fraction of the total content is of particular environmental concern. Notwithstanding this, *Bufo spinosus*, in terms of *BF*, seems to absorb more from water. The bioaccumulation factor is a rapid index of understanding the mobility of HMs from environmental matrices to organic tissues [55] and has proved to be useful for many animal species, including fish, mussels, and crustaceans [80]. The toad appears to be an accumulator for Fe and Zn regardless of the land use, even if this measurement does not permit the discrimination of their natural biological level from the anthropogenic assimilation as they are essential microelements [81,82]. On the other hand, for Pb and Cu, the studied amphibian acts like an accumulator for URB and IND, respectively.

This trend could be related to the different impacts of human activities on water, emphasized by the use of biological tools [83]. Thus, Pb and Cu can become markers of specific land use even if their concentrations are not as high as the copper in our study. The bioaccumulation factor for these elements shows an alert for the health status of the environment, as Pb and Cu are known to be hazardous to organisms and humans. Indeed, lead and copper impact the behavioral performance of organisms and act like endocrine disruptors [77,83]. Other studies tested the efficacy in utilization of amphibians for similar studies that confirmed the toxicity of Cu and Pb also for *Bufo arenarum* H. and *Lithobates chiricahuensis*, together with the difficulty to assess the negative effects of Fe and Zn in the health of amphibians [82,84,85].

5. Conclusions

The results of this research performed along the Medjerda River in the Souk-Ahras province of Northern Algeria highlight that agriculture, urbanization, and industrialization particularly affect riparian ecosystems through the release of HMs in terrestrial and aquatic compartments.

Specifically, IND, followed by URB land use, mostly impacted soil in terms of HMs such as Cu and Pb. Water quality is made a concern by a high concentration of Pb that exceeds limits suggested by WHO, although it is not possible to directly discriminate a different impact of the three land uses investigated as probably related to its dynamism and less conservativity. The environmental indices emphasize a moderate to considerable risk from agricultural to industrial and urban areas, and despite the fact that their concentrations do not exceed the regulatory limits, they recommend attention with regard to Zn. In terms of land use, toad responds like soil as IND is the most impactful management, whereas, for HMs, toad reflects the same trend of water as possibly related to the chemical affinity of the investigated elements. In addition, despite the single skin analyses not displaying any statistical difference among the land uses, the bioaccumulation index allows us to highlight that *Bufo spinosus* absorbs more HMs from water, especially Pb and Cu for URB and IND, as they are related to similarity in the chemical forms. However, this approach cannot discriminate if the toad also acts as an accumulator for Fe and Zn, as it is not possible to differentiate anthropogenic assimilation from natural biological levels. Anyway, *Bufo spinosus* turned out to be a good biosentinel for monitoring environmental quality in the studied riparian ecosystems, and the non-lethal technique of skin biopsy seems to be a powerful method able to obtain information from the animal without being invasive or killing the collected individuals.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/w16040538/s1, Figure S1: Total metal concentration (ppm) of Cu, Fe, Pb, and Zn reported as mean ± standard error in soil subjected to different land uses. Different letters show statistically significant differences (One-Way ANOVA, $p \leq 0.05$); Table S1: Means of raw values ± standard error for the bioaccumulation factors (BF) of heavy metals (Cu, Fe, Pb, and Zn) from water (*BFw*) and soil (*BFs*) to toads for sites subjected to different land use (AGR, URB, and IND).

Author Contributions: Conceptualization, A.D.M., G.G. and P.N.; methodology, software, A.D.M. and P.N.; validation, A.D.M., P.N., G.G., I.B., S.B., C.P. and N.G.; formal analysis, N.G., G.G., and P.N.; data curation, P.N. and A.D.M.; writing—original draft preparation, P.N., G.G. and A.D.M.; writing—review and editing, A.D.M., P.N., I.B., S.B., C.P. and G.G.; supervision, A.D.M. and G.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was conducted in strict accordance with European (Directive, 2010/63) legislation on the care and use of animals for scientific purposes. The skin biopsy was carried out only on adult male specimens that were later released.

Data Availability Statement: The data presented in this study are available upon request from the corresponding authors.

Acknowledgments: This work was performed in the framework of the international agreement (MoU) between the University of Mohamed Cherif Messaadia, Souk-Ahras (Algeria), and the University of Naples Federico II, Naples (Italy). We acknowledge all the people involved in this research for their technical support in the collection and analysis of toads, soil, and water and the unknown reviewers who helped us to improve this article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Calderon, M.R.; González, S.P.; Pérez-Iglesias, J.M.; Jofré, M.B. Anthropogenic impacts on rivers: Use of multiple indicators to assess environmental quality status. *Hydrobiologia* **2023**, *850*, 469–487. [CrossRef]
2. Rakshit, D.; Sahu, G.; Mohanty, A.K.; Satpathy, K.K.; Jonathan, M.P.; Murugan, K.; Sarkar, S.K. Bioindicator role of tintinnid (Protozoa: Ciliophora) for water quality monitoring in Kalpakkam, Tamil Nadu, south east coast of India. *Mar. Pollut. Bull.* **2017**, *114*, 134–143. [CrossRef]
3. Abas, A. A systematic review on biomonitoring using lichen as the biological indicator: A decade of practices, progress and challenges. *Ecol. Indic.* **2021**, *121*, 107197. [CrossRef]
4. Itziou, A.; Dimitriadis, V.K. Introduction of the land snail *Eobania vermiculata* as a bioindicator organism of terrestrial pollution using a battery of biomarkers. *Sci. Total Environ.* **2011**, *409*, 1181–1192. [CrossRef]
5. Deeb, M.; Desjardins, T.; Podwojewski, P.; Pando, A.; Blouin, M.; Lerch, T.Z. Interactive effects of compost, plants and earthworms on the aggregations of constructed Technosols. *Geoderma* **2017**, *305*, 305–313. [CrossRef]
6. Zina, V.; Ordeix, M.; Franco, J.C.; Ferreira, M.T.; Fernandes, M.R. Ants as bioindicators of riparian ecological health in catalonian rivers. *Forests* **2021**, *12*, 625. [CrossRef]
7. Coller, E.; Longa, C.M.O.; Morelli, R.; Zanoni, S.; Ippolito, M.C.C.; Pindo, M.; Cappelletti, C.; Ciutti, F.; Menta, C.; Zanzotti, R.; et al. Soil communities: Who responds and how quickly to a change in agricultural system? *Sustainability* **2022**, *14*, 383. [CrossRef]
8. Testi, A.; Fanelli, G.; Crosti, R.; Castigliani, V.; D'Angeli, D. Characterizing river habitat quality using plant and animal bioindicators: A case study of Tirino River (Abruzzo Region, Central Italy). *Ecol. Indic.* **2012**, *20*, 24–33. [CrossRef]
9. Panda, B.P.; Mohanta, Y.K.; Parida, S.P.; Pradhan, A.; Mohanta, T.K.; Patowary, K.; Wan Mahari, W.A.; Lam, S.S.; Ghfar, A.A.; Guerriero, G.; et al. Metal pollution in freshwater fish: A key indicator of contamination and carcinogenic risk to public health. *Environ. Pollut.* **2023**, *330*, 121796. [CrossRef] [PubMed]
10. De Agostini, A.; Cortis, P.; Cogoni, A. Monitoring of air pollution by moss bags around an oil refinery: A critical evaluation over 16 years. *Atmosphere* **2020**, *11*, 272. [CrossRef]
11. Özkök, E.A.; Çobanoğlu, G. Spatial evaluation of air quality by biomonitoring of toxic element accumulation in lichens in urban green areas and nature parks on the Anatolian side of Istanbul. *Environ. Monit. Assess.* **2023**, *195*, 908. [CrossRef]
12. Addesso, R.; Gonzalez-Pimentel, J.L.; D'Angeli, I.M.; De Waele, J.; Saiz-Jimenez, C.; Jurado, V.; Miller, A.Z.; Cubero, B.; Vigliotta, G.; Baldantoni, D. Microbial Community Characterizing Vermiculations from Karst Caves and Its Role in Their Formation. *Microb. Ecol.* **2021**, *81*, 884–896. [CrossRef]
13. Celletti, S.; Fedeli, R.; Ghorbani, M.; Loppi, S. Impact of starch-based bioplastic on growth and biochemical parameters of basil plants. *Sci. Total Environ.* **2023**, *856*, 159163. [CrossRef]
14. Monaci, F.; Ancora, S.; Paoli, L.; Loppi, S.; Franzaring, J. Air quality in post-mining towns: Tracking potentially toxic elements using tree leaves. *Environ. Geochem. Health* **2023**, *45*, 843–859. [CrossRef] [PubMed]
15. Piano, E.; Biagioli, F.; Nicolosi, G.; Coleine, C.; Poli, A.; Prigione, V.; Zanellati, A.; Addesso, R.; Varese, G.C.; Selbmann, L.; et al. Tourism affects microbial assemblages in show caves. *Sci. Total Environ.* **2023**, *871*, 162106. [CrossRef] [PubMed]
16. Rascio, I.; Gattullo, C.E.; Porfido, C.; Allegretta, I.; Spagnuolo, M.; Tiziani, R.; Celletti, S.; Cesco, S.; Mimmo, T.; Terzano, R. Fire-induced effects on the bioavailability of potentially toxic elements in a polluted agricultural soil: Implications for Cr uptake by durum wheat plants. *Environ. Sci. Pollut. Res.* **2023**, *30*, 6358–6372. [CrossRef] [PubMed]
17. Salo, J.V.B.; Solania, C.L. Article 2 Part of the Biodiversity Commons, Terrestrial and Aquatic Ecology Commons, and the Zoology Commons Recommended Citation Recommended Citation Salo. *J. Bioresour. Manag.* **2022**, *9*, 9–22.
18. Çömden, E.A.; Yenmiş, M.; Çakır, B. The Complex Bridge between Aquatic and Terrestrial Life: Skin Changes during Development of Amphibians. *J. Dev. Biol.* **2023**, *11*, 6. [CrossRef]

19. Gauthier, L.; Tardy, E.; Mouchet, F.; Marty, J. Biomonitoring of the genotoxic potential (micronucleus assay) and detoxifying activity (EROD induction) in the River Dadou (France), using the amphibian *Xenopus laevis*. *Sci. Total Environ.* **2004**, *323*, 47–61. [CrossRef] [PubMed]
20. Zhou, Q.; Zhang, J.; Fu, J.; Shi, J.; Jiang, G. Biomonitoring: An appealing tool for assessment of metal pollution in the aquatic ecosystem. *Anal. Chim. Acta* **2008**, *606*, 135–150. [CrossRef] [PubMed]
21. Thompson, D.G.; Wojtaszek, B.F.; Staznik, B.; Chartrand, D.T.; Stephenson, G.R. Chemical and biomonitoring to assess potential acute effects of Vision® herbicide on native amphibian larvae in forest wetlands. *Environ. Toxicol. Chem.* **2004**, *23*, 843–849. [CrossRef]
22. Guezgouz, N.; Parisi, C.; Boubsil, S.; Grieco, G.; Hana, S.A.; Guerriero, G. Heavy Metals Assessment in the Medjerda River Basin (Northeastern Algeria): A Preliminary Water Analysis and Toad Skin Biopsy. *Proc. Zool. Soc.* **2021**, *74*, 104–113. [CrossRef]
23. Napoletano, P.; Guezgouz, N.; Di Iorio, E.; Colombo, C.; Guerriero, G.; De Marco, A. Anthropic impact on soil heavy metal contamination in riparian ecosystems of northern Algeria. *Chemosphere* **2023**, *313*, 137522. [CrossRef]
24. Meek, R. Long-Term Changes in Four Populations of the Spiny Toad, *Bufo spinosus*, in Western France; Data from Road Mortalities. *Conservation* **2022**, *2*, 248–261. [CrossRef]
25. Zaimes, G.N.; Iakovoglou, V. Assessing riparian areas of Greece-an overview. *Sustainability* **2021**, *13*, 309. [CrossRef]
26. Groffman, P.M.; Bain, D.J.; Band, L.E.; Belt, K.T.; Brush, G.S.; Grove, J.M.; Pouyat, R.V.; Yesilonis, I.C.; Zipperer, W.C. Down by the riverside: Urban riparian ecology. *Front. Ecol. Environ.* **2003**, *1*, 315–321. [CrossRef]
27. Santolini, R.; Morri, E.; Pasini, G.; Giovagnoli, G.; Morolli, C.; Salmoiraghi, G. Assessing the quality of riparian areas: The case of River Ecosystem Quality Index applied to the Marecchia river (Italy). *Int. J. River Basin Manag.* **2015**, *13*, 1–16. [CrossRef]
28. Kooch, Y.; Tavakoli, M.; Akbarinia, M. Microbial/biochemical indicators showing perceptible deterioration in the topsoil due to deforestation. *Ecol. Indic.* **2018**, *91*, 84–91. [CrossRef]
29. Ndalilo, L.A.; Maranga, E.K.; Kirui, B.K. Land Use and Land Cover Change along River Lumi Riparian Ecosystem in Kenya: Implications on Local Livelihoods. *Open J. For.* **2021**, *11*, 206–221. [CrossRef]
30. Kindu, M.; Schneider, T.; Teketay, D.; Knoke, T. Drivers of land use/land cover changes in Munessa-Shashemene landscape of the south-central highlands of Ethiopia. *Environ. Monit. Assess.* **2015**, *187*, 452. [CrossRef] [PubMed]
31. Ali, M.M.; Rahman, S.; Islam, M.S.; Rakib, M.R.J.; Hossen, S.; Rahman, M.Z.; Kormoker, T.; Idris, A.M.; Phoungthong, K. Distribution of heavy metals in water and sediment of an urban river in a developing country: A probabilistic risk assessment. *Int. J. Sediment Res.* **2022**, *37*, 173–187. [CrossRef]
32. Abd Elnabi, M.K.; Elkaliny, N.E.; Elyazied, M.M.; Azab, S.H.; Elkhalifa, S.A.; Elmasry, S.; Mouhamed, M.S.; Shalamesh, E.M.; Alhorieny, N.A.; Abd Elaty, A.E.; et al. Toxicity of Heavy Metals and Recent Advances in Their Removal: A Review. *Toxics* **2023**, *11*, 580. [CrossRef]
33. Rahman, Z.; Singh, V.P. The relative impact of toxic heavy metals (THMs) (arsenic (As), cadmium (Cd), chromium (Cr)(VI), mercury (Hg), and lead (Pb)) on the total environment: An overview. *Environ. Monit. Assess.* **2019**, *191*, 419. [CrossRef] [PubMed]
34. Santorufo, L.; Memoli, V.; Panico, S.C.; Esposito, F.; Vitale, L.; Di Natale, G.; Trifuoggi, M.; Barile, R.; De Marco, A.; Maisto, G. Impact of Anthropic Activities on Soil Quality under Different Land Uses. *Int. J. Environ. Res. Public Health* **2021**, *18*, 8423. [CrossRef]
35. Joimel, S.; Cortet, J.; Jolivet, C.C.; Saby, N.P.A.; Chenot, E.D.; Branchu, P.; Consalès, J.N.; Lefort, C.; Morel, J.L.; Schwartz, C. Physico-chemical characteristics of topsoil for contrasted forest, agricultural, urban and industrial land uses in France. *Sci. Total Environ.* **2016**, *545–546*, 40–47. [CrossRef] [PubMed]
36. Belon, E.; Boisson, M.; Deportes, I.Z.; Eglin, T.K.; Feix, I.; Bispo, A.O.; Galsomies, L.; Leblond, S.; Guellier, C.R. An inventory of trace elements inputs to French agricultural soils. *Sci. Total Environ.* **2012**, *439*, 87–95. [CrossRef]
37. El Khalil, H.; Schwartz, C.; Elhamiani, O.; Kubiniok, J.; Morel, J.; Boularbah, A. Contribution of technic materials to the mobile fraction of metals in urban soils in Marrakech (Morocco). *J. Soils Sediments* **2008**, *8*, 17–22. [CrossRef]
38. Al-Kahtany, K.; Nour, Y.H.E.; El-Sorogy, A.S.; Alharbi, T. Ecological and health risk assessment of heavy metals contamination in mangrove sediments, Red Sea coast. *Mar. Poll. Bull.* **2023**, *192*, 115000. [CrossRef] [PubMed]
39. Berhail, S.; Katipoğlu, O.M. Spatiotemporal detection of abrupt change in trends of rainfall and dry and wet periods at different time scales: The case of the Medjerda basin in northeast Algeria. *Acta Geophys.* **2023**, *71*, 2497–2516. [CrossRef]
40. Dridi, A.; Tlili, A.; Fatnassi, S.; Hamrouni, H.; Gueddari, M. Effects of boron distribution on sugar beet crop yield in two Tunisian soils. *Arab. J. Geosci.* **2018**, *11*, 400. [CrossRef]
41. IUSS. *IUSS Working Group WRB: World Reference Base for Soil Resources 2014, Update 2015*; World Soil Resources Report No. 106; FAO: Rome, Italy, 2015.
42. Colombo, C.; Miano, T. *Metodi di Analisi Chimica del Suolo*; Pubblicità & Stampa: Bari, Italy, 2015.
43. De Marco, A.; Fioretto, A.; Giordano, M.; Innangi, M.; Menta, C.; Papa, S.; De Santo, A.V. C stocks in forest floor and mineral soil of two mediterranean beech forests. *Forests* **2016**, *7*, 181. [CrossRef]
44. Pribyl, D.W. A critical review of the conventional SOC to SOM conversion factor. *Geoderma* **2010**, *156*, 75–83. [CrossRef]
45. Lindsay, W.N.; Norwell, W.A. Development of a DTPA micronutrient soil test. *Agron. Abstr.* **1969**, *84*, 69–87.
46. Srodek, D.; Rahmonov, O. The Properties of Black Locust *Robinia pseudoacacia* L. to Selectively Accumulate Chemical Elements from Soils of Ecologically Transformed Areas. *Forests* **2022**, *13*, 7. [CrossRef]

47. Mueller, L.; Schindler, U.; Mirschel, W.; Graham Shepherd, T.; Ball, B.C.; Helming, K.; Rogasik, J.; Eulenstein, F.; Wiggering, H. As-sessing the productivity function of soils. A review. *Agron. Sustain. Dev.* **2010**, *30*, 601–614. [CrossRef]
48. Wedepohl, K.H. The composition of the continental crust. *Geochim. Cosmochim. Acta* **1995**, *59*, 1217–1232. [CrossRef]
49. Okedeyi, O.O.; Dube, S.; Awofolu, O.R.; Nindi, M.M. Assessing the enrichment of heavy metals in surface soil and plant (*Digitaria eriantha*) around coal-fired power plants in South Africa. *Environ. Sci. Pollut. Res.* **2014**, *21*, 4686–4696. [CrossRef]
50. Hakanson, L. Ecological risk index for aquatic pollution control, a sedimentological approach. *Water Res.* **1980**, *14*, 975–1001. [CrossRef]
51. Rahmonov, O.; Sobala, M.; Srodek, D.; Karkosz, D.; Pytel, S.; Rahmonov, M. The spatial distribution of potentially toxic elements in the mountain forest topsoils (the Silesian Beskids, southern Poland). *Sci. Rep.* **2024**, *14*, 338. [CrossRef]
52. Ahamad, M.I.; Song, J.; Sun, H.; Wang, X.; Mehmood, M.S.; Sajid, M.; Su, P.; Khan, A.J. Contamination level, ecological risk, and source identification of heavy metals in the hyporheic zone of the Weihe river, China. *Int. J. Environ. Res. Public Health* **2020**, *17*, 1070. [CrossRef]
53. Heyer, R.; Donnelly, M.A.; Foster, M.; Mcdiarmid, R. *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*; Smithsonian Institution: Washington, DC, USA, 2014.
54. D'Errico, G.; Vitiello, G.; De Tommaso, G.; Abdel-Gawad, F.K.; Brundo, M.V.; Ferrante, M.; De Maio, A.; Trocchia, S.; Bianchi, A.R.; Ciarcia, G.; et al. Electron Spin Resonance (ESR) for the study of Reactive Oxygen Species (ROS) on the isolated frog skin (*Pelophylax bergeri*): A non-invasive method for environmental monitoring. *Environ. Res.* **2018**, *165*, 11–18. [CrossRef]
55. Kuziemska, B.; Wysokinski, A.; Klej, P. The Content, Uptake and Bioaccumulation Factor of Copper and Nickel in Grass Depending on Zinc Application and Organic Fertilization. *Agriculture* **2023**, *13*, 1676. [CrossRef]
56. Ionescu, P.; Radu, V.M.; Deak, G.; Ciobotaru, I.E.; Marcu, E.; Diacu, E.; Pipirigeanu, M. Bioaccumulation of potentially toxic elements in fish species from aquatic environments located in crowded areas of southern Romania. *Tech. Rom. J. Appl. Sci. Technol.* **2019**, *1*, 53–58. [CrossRef]
57. Lin, H. Three Principles of Soil Change and Pedogenesis in Time and Space. *Soil Sci. Soc. Am. J.* **2011**, *75*, 2049–2070. [CrossRef]
58. Akhtar, N.; Syakir Ishak, M.I.; Bhawani, S.A.; Umar, K. Various natural and anthropogenic factors responsible for water quality degradation: A review. *Water* **2021**, *13*, 2660. [CrossRef]
59. Oyeyiola, A.O.; Davidson, C.M.; Olayinka, K.O.; Alo, B.I. Fractionation and ecotoxicological implication of potentially toxic metals in sediments of three urban rivers and the Lagos Lagoon, Nigeria, West Africa. *Environ. Monit. Assess.* **2014**, *186*, 7321–7333. [CrossRef] [PubMed]
60. Guasmi, I.; Kherici-Bousnoubra, H.; Kherici, N.; Hadji, F. Assessing the organic pollution of surface water of Medjerda watershed (NE Algeria). *Environ. Earth Sci.* **2010**, *60*, 985–992. [CrossRef]
61. Ben Ayed, L.; Horry, M.; Sabbahi, S.; Nouiri, I.; Karanis, P. Physico-chemical quality of the Medjerda River in Tunisia and suitability for irrigation during the moist and the dry seasons. *Bull. Soc. R. Sci. Liege* **2022**, *91*, 23–43. [CrossRef]
62. Fayiga, A.O.; Ipinmoroti, M.O.; Chirenje, T. *Environmental Pollution in Africa*; Springer: Dordrecht, The Netherlands, 2018; Volume 20, ISBN 1066801698944.
63. Noulas, C.; Tziouvalekas, M.; Karyotis, T. Zinc in soils, water and food crops. *J. Trace Elem. Med. Biol.* **2018**, *49*, 252–260. [CrossRef] [PubMed]
64. Shifaw, E. Review of heavy metals pollution in China in agricultural and urban soils. *J. Health Pollut.* **2018**, *8*, 180607. [CrossRef] [PubMed]
65. Jdid, E.A.; Blazy, P.; Kamoun, S.; Guedria, A.; Marouf, B.; Kitane, S. Environmental impact of mining activity on the pollution of the Medjerda River, north-west Tunisia. *Bull. Eng. Geol. Environ.* **1999**, *57*, 273–280. [CrossRef]
66. AFNOR. Qualité des sols. In *Recueil de Normes Françaises*; AFNOR: Paris, France, 1996.
67. Bartoli, G.; Papa, S.; Sagnella, E.; Fioretto, A. Heavy metal content in sediments along the Calore river: Relationships with physical-chemical characteristics. *J. Environ. Manag.* **2012**, *95*, S9–S14. [CrossRef]
68. World Health Organization (WHO). *Evaluating Household Water Treatment Options: Health-Based Targets and Microbiological Performance Specifications*; World Health Organization: Geneva, Switzerland, 2011.
69. Prokof'eva, T.V.; Kiryushin, A.V.; Shishkov, V.A.; Ivannikov, F.A. The importance of dust material in urban soil formation: The experience on study of two young Technosols on dust depositions. *J. Soils Sediments* **2017**, *17*, 515–524. [CrossRef]
70. Boubehziz, S.; Khanchoul, K.; Benslama, M.; Benslama, A.; Marchetti, A.; Francaviglia, R.; Piccini, C. Predictive mapping of soil organic carbon in Northeast Algeria. *Catena* **2020**, *190*, 104539. [CrossRef]
71. Adegbidi, H.G.; Briggs, R.D.; Volk, T.A.; White, E.H.; Abrahamson, L.P. Effect of organic amendments and slow-release nitrogen fertilizer on willow biomass production and soil chemical characteristics. *Biomass Bioenergy* **2003**, *25*, 389–398. [CrossRef]
72. Panico, S.C.; Esposito, F.; Memoli, V.; Vitale, L.; Polimeno, F.; Magliulo, V.; Maisto, G.; De Marco, A. Variations of agricultural soil quality during the growth stages of sorghum and sunflower. *Appl. Soil Ecol.* **2020**, *152*, 103569. [CrossRef]
73. Tao, H.; Bobaker, A.M.; Ramal, M.M.; Yaseen, Z.M.; Hossain, M.S.; Shahid, S. Determination of biochemical oxygen demand and dissolved oxygen for semi-arid river environment: Application of soft computing models. *Environ. Sci. Pollut. Res.* **2019**, *26*, 923–937. [CrossRef]
74. Yadav, R.K.; Goyal, B.; Sharma, R.K.; Dubey, S.K.; Minhas, P.S. Post-irrigation impact of domestic sewage effluent on composition of soils, crops and ground water—A case study. *Environ. Int.* **2002**, *28*, 481–486. [CrossRef]

75. Kolář, L.; Klimeš, F.; Ledvina, R.; Kužel, S. A method to determine mineralization kinetics of a decomposable part of soil organic matter in the soil. *Plant Soil Environ.* **2003**, *49*, 8–11. [CrossRef]
76. Guerriero, G.; Brundo, M.V.; Labar, S.; Bianchi, A.R.; Trocchia, S.; Rabbito, D.; Palumbo, G.; Abdel-Gawad, F.K.; De Maio, A. Frog (*Pelophylax bergeri*, Günther 1986) endocrine disruption assessment: Characterization and role of skin poly(ADP-ribose) polymerases. *Environ. Sci. Pollut. Res.* **2018**, *25*, 18303–18313. [CrossRef] [PubMed]
77. Guerriero, G.; D'Errico, G.; Di Giaimo, R.; Rabbito, D.; Olanrewaju, O.S.; Ciarcia, G. Reactive oxygen species and glutathione antioxidants in the testis of the soil biosentinel *Podarcis sicula* (Rafinesque 1810). *Environ. Sci. Pollut. Res.* **2018**, *25*, 18286–18296. [CrossRef]
78. Kaufmann, K.; Dohmen, P. Adaption of a dermal in vitro method to investigate the uptake of chemicals across amphibian skin. *Environ. Sci. Eur.* **2016**, *28*, 10. [CrossRef]
79. Singh, A.; Sharma, A.; Verma, R.K.; Chopade, R.L.; Pandit, P.P.; Nagar, V.; Aseri, V.; Choudhary, S.K.; Awasthi, G.; Awasthi, K.K. Heavy Metal Contamination of Water and Their Toxic Effect on Living Organisms. In *The Toxicity of Environmental Pollutants*; IntechOpen: London, UK, 2022.
80. DeForest, D.K.; Brix, K.V.; Adams, W.J. Assessing metal bioaccumulation in aquatic environments: The inverse relationship between bioaccumulation factors, trophic transfer factors and exposure concentration. *Aquat. Toxicol.* **2007**, *84*, 236–246. [CrossRef]
81. Dobrovoljc, K.; Falnoga, I.; Žnidarič, M.T.; Mazej, D.; Ščančar, J.; Bulog, B. Cd, Cu, Zn, Se, and metallothioneins in two amphibians, *necturus maculosus* (Amphibia, Caudata) and *Bufo bufo* (Amphibia, Anura). *Biol. Trace Elem. Res.* **2012**, *150*, 178–194. [CrossRef]
82. Da Silva Veronez, A.C.; Salla, R.V.; Baroni, V.D.; Barcarolli, I.F.; Bianchini, A.; dos Reis Martinez, C.B.; Chippari-Gomes, A.R. Genetic and biochemical effects induced by iron ore, Fe and Mn exposure in tadpoles of the bullfrog *Lithobates catesbeianus*. *Aquat. Toxicol.* **2016**, *174*, 101–108. [CrossRef] [PubMed]
83. Huang, M.Y.; Duan, R.Y.; Ji, X. Chronic effects of environmentally-relevant concentrations of lead in *Pelophylax nigromaculata* tadpoles: Threshold dose and adverse effects. *Ecotoxicol. Environ. Saf.* **2014**, *104*, 310–316. [CrossRef] [PubMed]
84. Herkovits, J.; Alejandra Helguero, L. Copper toxicity and copper-zinc interactions in amphibian embryos. *Sci. Total Environ.* **1998**, *221*, 1–10. [CrossRef] [PubMed]
85. Calfee, R.D.; Little, E.E. Toxicity of Cadmium, Copper, and Zinc to the Threatened Chiricahua Leopard Frog (*Lithobates* [Rana] *chiricahuensis*). *Bull. Environ. Contam. Toxicol.* **2017**, *99*, 679–683. [CrossRef] [PubMed]