CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE
FACULTY OF ENVIRONMENTAL SCIENCES

MASTER THESIS

Accumulation of toxic metals in sediment and biota of the Lužické mountains springs

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Faculty of Environmental Sciences

DIPLOMA THESIS ASSIGNMENT

Rosmery Elizabeth Amalia Noriega Silva

Land and Water Management

Thesis title

Accumulation of toxic metals in sediment and biota of the Lužické Mountains springs

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Objectives of thesis

The goal of the thesis is to assess occurrence of toxic metals in sediment and biota of springs located in the Lužické Mountains area. The thesis will evaluate the risk related to metals occurrence and compare the role of geological background on metal distribution in the aquatic environment.

Methodology

1)Collection of sediment and macroinvertebrates in 20 streams (the sampling will be done twice a year)

2)Microwave digestions of sediment and biota samples (method US EPA 3052)

3)Sequential extraction and identification of geochemical fraction important for metals binding

4)Analyses of metal contain in sediment and biota samples

5)Identification of the risk – application distribution coefficient, biota sediment accumulation index, hazard quotient,

6)Assessment of the impact of geological background on metal occurrence

The proposed extent of the thesis

75

Keywords

toxic metals; biota; sediment; spring; accumulation; Lužické mountains

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Recommended information sources

Barnthouse LW, DeAngelis DL, Gardner RH, O´Neill RV, Suter GW II and Vaughan DS (1982) Methodology for risk environmental risk analysis. ORNL/TM/8167. Oak Ridge, TN. Oak Ridge National Laboratory
Rand GM (1995) Fundamentals of aquatic toxicology. Effects. Environmental fate and risk assessment.,2nd edn. CRC Press, Boca Raton, USA, 1148 pp
Stumm Werner and Morgan James J. 1995. AQUATIC CHEMISTRY: Chemical Equilibria and Rates in Natural Waters, 3rd Edition. United State of America. ISBN: 978-0-471-51185-4. 1040 pages.
Tessier A, Campbell PGC, Bisson M (1979) Sequential extraction procedure for the speciation of trace metals. Anal Chem 51:844-851
White William M. 2013. GEOCHEMISTRY. Department of Earth & Atmospheric Science. Ithaca, New York, USA. 672 pages. 211-258 pg.
☐ Carvalho Alexandra and Steven J. Schropp. 2002. Development of an Interpretive Tool for Assessment of Metal Enrichment in Florida Freshwater Sediment. Florida Department of Environmental Protection

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Prague on 06. 04. 2017

DECLARATION

I declare that this thesis entitled “Accumulation of toxic metals in sediment and biota of the Lužické mountains springs” is the result of my own research under guidance of RNDr. Dana Komínková, Ph.D. and Ing. Melchor Herbert Dolmos Castro, except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

April 18, 2017 Rosmery Noriega Silva

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Finally, special thanks go to Aldo. He accompanied me from the beginning and helped me enormously. He is my greatest partner!

ABSTRACT

The accumulation of Cd, Cu, Zn and Pb in water, sediment and biota collected along Lužické mountains springs were determined using flame 55 Atomic Absorption Spectrometer (F55AAS) and graphite tube atomizer AA (GTA 120) (Agilent technologies). Sediments were subjected to both sequential extraction and pseudototal digestion procedures. Low pH, electrical conductivity, OD, and temperature were found in the autumn season than spring season. Near neutral pH (5.49 - 8.02) was found in 11 from 20 springs during spring season; while in the autumn season, acid pH (4.62 - 6.80) was observed in 15 from 18 springs. During spring season conductivity ranged from 78.7 - 762 µS/cm, the temperature ranged from 7.3 - 14.1ºC and OD ranged from 4.11 - 10.83 mg.l^{-1}. During autumn season conductivity ranged from 93 - 907 µS/cm, the temperature ranged from 6.6 - 11.6ºC and OD ranged from 3.19 - 11.28 mg.l^{-1}. The toxic metal concentrations in water ranged from 0.0001 - 0.0015 mg/l Cu, 0.0002 - 0.0116 mg/l Zn, 0.0000 - 0.0002 mg/l Pb and 0.0000 - 0.0004 mg/l Cd; in sediment 0.00 - 91.70 mg/kg Cu, 9.15 - 134.71 mg/kg Zn, 0.00 - 83.67 mg/kg Pb and 0.01 - 3.56 mg/kg Cd and in biota 4.86 - 53.52 mg/kg Cu, 46.82 - 191.44 mg/kg Zn, 0.00 - 6.93 mg/kg Pb and 0.01 - 5.99 mg/kg Cd. According to the logarithmic distribution coefficient – log Kd, the metal most easily released from the sediment is Cu (2.8) in BS1017, log Kd ranged from 2.80 - 5.58 Cu, 3.53 - 5.53 Zn, 3.39 - 4.88 Cd and 5.01 - 6.62 Pb, log Kd values >4 reflect lower availability, which means lower migration potential into liquid phase. According to HQ, sediments of springs do not seem to be significantly loaded by toxic metals, with the exception of Pb, HQ-TEC > 1 were found in ES1014, ES1009, EN1021, EN1011, EN1004, DN1012 and DN1010, and HQ-PEC > 0.5 was found in EN1021. EN1022 was considered the most contaminated spring with toxic metals Cd (3.56 mg.kg^{-1}), Zn (134.71 mg.kg^{-1}), Cu (43.48 mg.kg^{-1}) and Pb (62.37 mg.kg^{-1}), specially Cu, 13 times more than the background values; however, mobility factor – MF is significantly very high for Cd - 91%, Cu - 73.60%, Zn - 36.85% and Cd - 4.05%. Biota sediment accumulation factor – BSAF ranged from 0.96 - 16.44 Cu, 1.11 - 16.89 Zn, 0.87 - 81.89 Cd and 0.02 - 0.37 Pb, BSAF recorded the highest values Cd accumulation. The following order of bioavailability was found with the toxic metals Cd > Zn > Cu > Pb.

key words:

Toxic metals, biota, sediment, spring, accumulation, Lužické mountains

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I. Introduction:

Springs are an interface between surface and groundwater (Williams, 1991). They provide unique habitats for many rare species (Lindegaard et al., 1998) and for relict species that have survived in an environment of rather stable ambient conditions (Von Fumetti, 2008 ex. Hynes, 1970; Ito, 1998). At their sources (orifices, points of emergence), the physical geomorphic template allows some springs to support numerous microhabitats and large arrays of aquatic, wetland, and terrestrial plant and animal species (Springer & Stevens, 2009). In the other hand, springs are rather limited in size, which makes them very vulnerable to anthropogenic impacts (Orendt, 2000) and leads to a relatively low number of species found in individual springs (Lindegaard et al., 1998; Von Fumetti, 2008).

Metals have received particular attention among other non-degradable toxic chemicals because of their adverse effects on the environment, organisms, human health and especially in sediment, water quality and some aquatic life forms. Sediments accumulate higher levels of hazardous trace elements than water, causing serious problems due to their toxicity and bioaccumulation (Morillo et al., 2007; Seen et al., 2008). The toxicological effect of metals has not been well understood, especially if they occur in mixture of metals or are present with other chemical components such as nutrients or sulfides (Komínková & Nábělková, 2007). It is necessary to identify and quantify the mode of occurrence in which a metal is present in sediment to gain a more precise understanding of the potential and actual impacts of the elevated level of metals in sediments (Singh et al., 2005). Extensive studies on the accumulation of toxic metals on springs are needed to gather information about their potential risk in the environment for the prevention and effective management of spring ecosystems. The research is focused on the assessment of the chemical status of springs and the importance of toxic metal accumulation in aquatic ecosystems as a part of the ecological status assessment.

The aim of the research is to assess the occurrence of toxic metals in sediment and biota of springs located in the Lužické Mountains area. The research will evaluate the risk related to metals occurrence and compare the role of geological background on metal distribution in the aquatic environment.

II. Literature Review:

2.1. SPRINGS

Springs are unique ecosystems at the interface between groundwater and surface water (Wetzel & Webb, 2007 ex. Webb et al., 1998) and between the terrestrial and the aquatic habitat (Cantonati et al., 2006). They are an ecotone providing their own characteristic environment for the organisms living in and around springs (Lindegaard et al., 1998; Von Fumetti, 2008). In contrast to the rhithral and potamal of rivers and streams they provide relatively stable abiotic conditions with small temperature amplitudes, little impact of nutrients and also a relatively stable discharge regime (Von Fumetti, 2008 ex. Van der Kamp, 1995). Karst springs, which are often intermittent springs, are exceptional, responding quickly to strong rainfalls and drought periods (Zollhöfer et al., 2000). Despite their ubiquitous distribution on earth and their special characteristics, making them "natural laboratories" (Von Fumetti, 2008 ex. Odum, 1971), fewer perennial, natural springs are now found.

A unique characteristic of springs is that they form a three-way ecotone where groundwater, surface water and terrestrial ecosystems interact (Barquín & Death, 2009; Barquín & Scarsbrook, 2007). The location of springs at the interface between several distinct ecosystems creates a heterogeneous mosaic of aquatic, semi-aquatic and semi-terrestrial microhabitats, which has led many ecologists to suggest that springs are ''hot spots'' for aquatic biodiversity (Barquín & Death, 2009 ex. Cantonati et al., 2006; Scarsbrook et al., 2007). Habitat heterogeneity is usually higher in helocrene springs, where water emerges in a swampy area, medium in rheocrene springs, where water emerges directly into a stream channel, and lower in limnocrene springs, where water emerges into a pool (Lindegaard et al. 1998; Cantonati et al. 2006; Barquín & Death, 2009)

After comprehensive studies at the beginning of the 20th century (e.g. Steinmann, 1915; Thienemann, 1926; Geijskes, 1935) the interest in springs decreased, aside studies on energy fluxes and trophic relationships in spring ecosystems as excellent models for ecosystems in general (Von Fumetti, 2008 ex. Odum, 1957). Previous investigations assumed that springs are habitats with nearly stable physical and chemical conditions (Von Fumetti, 2008 ex. Odum, 1957; van der Kamp, 1995). Thermal stability has been thought to be one of the main characteristics of springs and the reason for the presence of cold stenothermic animals (Von Fumetti, 2008 ex. Illies, 1952; Erman & Erman, 1995 ex.). However, springs differ in their geomorphological features and their water supply from deep or shallow groundwater. Conditions in springs also change on a temporal scale. Within a spring and between springs, these variabilities lead to a patchy environment with fluctuating conditions for small, isolated populations as described in the patch dynamics concept (Von Fumetti, 2008 ex. Pickett & White, 1985).

A number of reviews have been published in the 1990s (Williams et al. 1990; Ferrington, 1995; Botosaneanu 1998), which has helped to understand the ecology of springs and the importance of some key factors controlling the composition and structure of their biological communities, especially benthic invertebrate assemblages (Barquín & Death, 2009).

2.1.1. Spring origin in Europe

Spring research seriously started in Europe with the limnologist August Thienemann, an important researcher for the whole limnology. His trips to the isle of Rügen in the Baltic Sea (1925, 1926) and his research in the German low mountain ranges(1912) and lowlands (1923, 1926) were the first comprehensive studies on springs and their springbrooks (Von Fumetti, 2008). Thienemann was also the first, who invented the theory, that some spring species are glacial relicts that survived in springs because of their stable temperature conditions (Von Fumetti, 2008). Another important study on springs and springbrooks was conducted by Steinmann (1915) (Von Fumetti, 2008). He invented the spring typology of rheocrenes as “Sturzquellen” and limnocrenes as “Tümpelquellen”, Thienemann (1924) then added the helocrenes, the typical swamp springs from the north german lowlands (Von Fumetti, 2008). This “trinity” is still the most common ecomorphological spring typology, Thienemann’s student and successor Joachim Illies also conducted comprehensive studies on river systems including springs and springbrooks (Von Fumetti, 2008). He stated that the spring ends where the annual water temperature amplitude exceeds five degrees (Von Fumetti, 2008). This resulted in the famous zonation concept with the krenal as the spring region, the rhithral as the upper river section of the lower mountain range regions and the potamal as the lower river section of the lowlands. He also invented the terms eucrenal as the spring source and hypocrenal as the springbrook (Von Fumetti, 2008 ex. Illies &

Botosaneanu, 1963). After a time of little interest in spring ecosystems, a new period of spring research activity started in the late 1980's (Von Fumetti, 2008).

Due to a new environmental awareness the pollution of springs, their conservation and restoration and the evaluation of springs came in the focus of wider interest (Von Fumetti, 2008 ex. Verdonschot, 1995; Zollhöfer, 1997; Schindler, 2004). Despite a decrease of interest in our new century some spring research groups in Europe are still active and start investigating springs all over Europe again (e.g. Hoffsten & Malmqvist, 2000; Habdija et al., 2002; Smith et al., 2003; Ilmonen & Paasivirta, 2005; Mori & Brancelj, 2006) (Von Fumetti, 2008).

2.1.2. Classification

Much effort in the last decade was exerted to establish a classification of springs and to identify environmental variables that determine the composition of spring faunal communities. Researchers have agreed on the major distinction between limnocrene, rheocrene and helocrene springs, as they were distinguished by Thienemann (1924) (Blackwood et al., 1995; Ferrington, 1998; Hahn 2000; Von Fumetti & Nagel, 2011). Since then, many other spring typologies for different mid-European regions based on ecomorphological features (Von Fumetti, 2008 ex. Beyer, 1932; Schwoerbel, 1959; Gerecke, 1991), physicochemical (Hahn, 2000) or chemical factors (Roca, 1990; Williams et al., 1997) have been developed. And also some attempts have been made to include spring invertebrate communities (Glazier & Gooch 1987; Roca & Castillo 1993; Lindegaard et al. 1998; Hahn 2000; Meyer et al. 2003; Ilmonen & Paasivirta 2005; Barquín and Death 2007)

Helocrene

Helocrene springs usually emerge in a diffuse fashion in cienega (marshy, wet meadow) settings (Fig. 1 and 2); where A (aquifer), I (impermeable stratum) and S (spring source). Hynes (1970) distinguished these types of springs as different from the limnocrene type springs described by Bornhauser (1913) halophytes (Springer & Stevens, 2009). What are described as soap holes ormud springs in Alberta also are examples of helocrenes. A soap hole or mud spring is "a part of the land surface characterized by a local weakness of limited extent underlain by a mixture of sand, silt, clay, and water" (Springer & Stevens, 2009 ex.Toth, 1966). The formation of these springs is similar to that of quicksand. In the semi-arid regions of Alberta, Canada where these occur, groundwater discharge is typically saline, leading to the occurrence of halophytes (Springer & Stevens, 2009).

Other helocrenes may have fresh water, but low oxygen concentrations, and support species characteristic of wetlands, or they may have thermal waters and primarily support bacteria. Other helocrene springs may have hypersaline water and support marine relict taxa that may occur far inland on continents at great distances from the ocean (Grasby & Londry 2007). The wetland continuum of Euliss et al. (2004) provides further classification of helocrenes (Springer & Stevens, 2009).

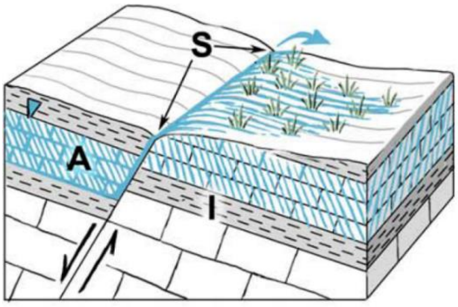


Fig. 1. Helocrene. Modified from Meinzer (1923). Springer et al., 2008.

Fig. 2. Helocrene, soap hole, Elk Island National Park, Alberta, Canada. Source: Springer et al., 2008.

Limnocrene

Limnocrene springs occur where discharge from confined or unconfined aquifers emerge as one or more lentic pools (Figs. 3 and 4). The term was first used by Bornhauser (1913) and then reinforced by Hynes (1970) (Springer & Stevens, 2009). Limnocrene springs exist in both the wetland continuum and groundwater dependent ecosystems classification systems. Although limnocrene springs may have a pond and aquatic species, their relatively uniform temperature and chemistry may cause different species to be present than in an adjacent surface-water dominated water body (Springer & Stevens, 2009). Montezuma Well in central Arizona is a limnocrene pool in a collapsed carbonate mound spring; the harsh, uniform water chemistry there appears to support the highest concentration of endemic species of any point in North America (Stevens, 2007).

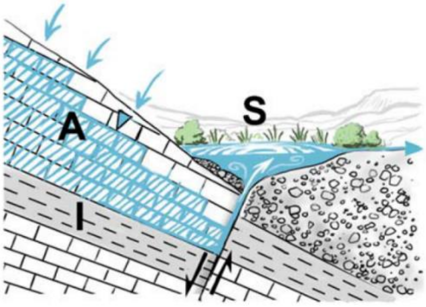


Fig. 3. Limnocrene. Modified from Meinzer (1923). Springer et al., 2008.

Fig. 4. Limnocrene, Grassi Lakes, Alberta, Canada. Source: Springer et al., 2008.

Rheocrene

The term rheocrene was first coined by Bornhauser (1913) to describe springs where discharge emerges as flowing streams (Figs. 5 and 6). Spring-fed streams are also referred to as springbrooks or spring runs. The term was continued as a special habitat of running waters by Hynes (1970) because of the relatively uniform temperature and the de-oxygenated groundwater contribution to the stream (Springer et al., 2008).

Springer et al. (2008) further recognized that there is a continuum between channels which are springs discharge dominated and those that are dominated by surface runoff. These longitudinal changes in flood-related disturbance, water quality, and geomorphology strongly direct evolutionary processes. Springflow-dominated springs may be sufficiently stable habitats to allow for evolutionary micro adaptation, and ultimately speciation, whereas surface flow-dominated systems are typically occupied by weedy, generalist species (McCabe, 1998).

The different types of channels along this continuum are distinctively different, in turn influencing the types of micro habitats that exist in them (Griffiths et al. 2008; Springer et al., 2008).

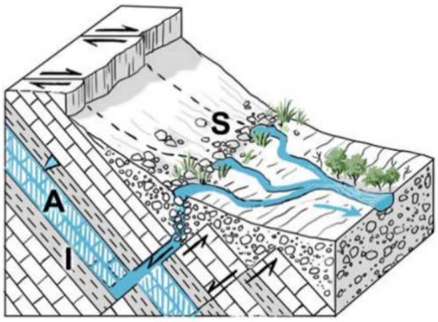


Fig. 5. Rheocrene. Modified from Meinzer (1923). Springer et al., 2008.

Fig. 6. Rheocrene, Pheasant Branch Spring, Wisconsin, US. Source: Springer et al., 2008.

Other types of springs:

Another prominent classification system that includes springs is for groundwater dependent ecosystems (GDE). GDE classification tends to focus more on vegetation components of the ecosystem because of the paucity of invertebrate data. The three classes described are: (1) aquifer and cave ecosystems, (2) all ecosystems dependent on the surface expression of flow, and (3) all ecosystems dependent on the subsurface presence of groundwater (Eamus & Froend, 2006; Springer et al., 2008). Springer et al. (2008) expanded the historical schemes described by Meinzer (1923), to include 12 spheres of discharge of springs, including: (1) springs that emerge in caves, (2) exposure springs, (3) artesian fountains, (4) geysers, (5) gushets, (6) contact hanging gardens, (7) helocrene wet meadows, (8) hillslope springs, (9) hypocrene buried springs, (10) limnocrene surficial lentic pools, (11) mound forms, and (12) rheocrene lotic channel floors (Springer et al., 2008).

An integrated springs classification system should include the major physical, biological, and socio-cultural variables. Such a classification system will permit assessment of the distribution of different kinds of springs ecosystems, thereby improving resource inventory and development of conservation and restoration strategies (Sada & Vinyard 2002; Perla & Stevens 2008; Springer et al., 2008). Alfaro and Wallace (1994) and Wallace and Alfaro (2001) updated and reviewed the historical springs classification schemes of Fuller (1904), Keilhack (1912), Bryan (1919), Meinzer (1923), Clarke (1924), Stiny (1933), and others; Meinzer's (1923) classification system has been the most persistently recognized (Springer et al., 2008). Although Meinzer's (1923) scheme has been widely used, it is not comprehensive (Springer et al., 2008). Clarke (1924) classified mineral springs waters according to their principal anion. Consequently the main types include chloride, sulfate, carbonate and acid waters. There are, in adition silicate, borate, nitrate, sulfide, and phosphate waters and water of mixed character. It is evident that this classification is only approximate and rather flexible (Rankama & Sahama, 1968).

Clarke (1924) considered three criteria to be most important for springs classification: geologic origin, physical properties, and geochemistry (Springer et al., 2008). Other classifications have been developed for specific types of geomorphology such as karst geomorphology (free draining, dammed, or confined springs) (Ford & Williams, 2007) or classification of karst springs by eight attributes: flow duration, reversing flow, conduit type at spring, geology, topographic position, relationship to bodies of surface water, distributaries, recharge, chemistry, culture/exploitation (Springer et al., 2008). Springs, particularly those in arid regions, are renowned as hotspots of biological and cultural diversity, and the presence of endangered or unique species and ethnological and historic resources often greatly influences their management. Therefore, ecological and cultural variables also relevant to springs classification include size, spatial isolation, microhabitat distribution, paleontological resources, the presence of rare or endemic biota, archeological or traditional cultural resources, and a springs’ context to surrounding ecosystems (Springer & Stevens, 2009).

2.1.3. Water quality and spring-water chemistry

Water quality is a term used here to express the suitability of water to sustain various uses or processes. Any particular use will have certain requirements for the physical, chemical or biological characteristics of water; for example limits on the concentrations of toxic substances for drinking water use, or restrictions on temperature and pH ranges for water supporting invertebrate communities (Chapman & Kimstach, 1996 in UNESCO et al., 1996). Consequently, water quality can be defined by a range of variables which limit water use. Although many uses have some common requirements for certain variables, each use will have its own demands and influences on water quality (Chapman & Kimstach, 1996 in UNESCO et al., 1996). Maintaining good water quality in water bodies inhabited by fish and other biota is a basic requirement of aquatic habitat integrity. The list of specific constituents generally includes water temperature, turbidity (suspended solids), dissolved gases (e.g., oxygen), nutrients, metals, inorganic and organic chemicals, and pH (Spence et al., 1996; Marsalek et al., 2005). While many of these constituents occur naturally and their concentrations exhibit certain ranges and fluctuations, large perturbations of such cycles may impair the performance of biological communities (Yoder, 1989; Marsalek et al., 2005).

Water quality is affected by a wide range of natural and human influences. The most important of the natural influences are geological, hydrological and climatic conditions, since these affect the quantity and the quality of available water (Chapman & Kimstach, 1996 in UNESCO et al., 1996). Contaminants can impair water quality and affect water use; a contaminant is an undesirable substance in water that either is not normally present or is a naturally occurring substance at an unusually high concentration (Vandas et al., 2002).

According to Vandas et al. (2002), contaminants can be divided into four general classes: sediment and natural organic materials, nutrients, bacteria, and toxic substances. These can enter water by either point or non-point sources. Point sources contribute contaminants at a discrete site, such as the outflow from a pipe, ditch, tunnel, well, concentrated animal-feeding operation, or floating craft (Vandas et al., 2002). Non-point sources contribute contaminants from a broad area; as a result, such sources are not as easily identified or controlled as point sources. Non-point sources include the atmosphere, agricultural areas, golf courses, residential developments, roads, parking lots, and contributions from groundwater along lengthy reaches of streams (Vandas et al., 2002).

Water supplying springs come mostly from precipitation, which has to pass through the plant cover on its way to the soil and bedrock (Malek & Krakowian, 2012). According to Williams (1991) springs are the most predictable freshwater habitats. Springs influence the downstream spring brook. For example, close to the spring annual temperatures of alpine rheocrenes vary only 1-2 °C over the annual cycle and alpine or arctic spring brooks remain ice-free throughout the winter (Malard et al., 1999; Ward, 1994; Döring, 2002). Usually, springs tend to have relatively constant concentrations of dissolved minerals (Van der Kamp, 1995; Döring, 2002), even during periods of rainfall, snowmelt and drought, in contrast to surface fed streams. The solute concentrations depend on the geology of the passed strata (Freeze & Cherry, 1979; Döring, 2002). According to Mattson et al. (1995), the water chemistry of springs is usually very different compared to surface waters in the same region because retention of infiltrated water may range from days to many years (Van der Kamp, 1995; Döring, 2002). In response to surface water infiltration, water chemistry and discharge of the spring will change. The extent of changes depends on precipitation, snowmelt, and the morphology of the spring brook channel and catchment area (Ryan & Meiman, 1996; Döring, 2002). But the relationships between precipitation events and spring discharge and chemistry are complex (McCabe, 1998; Döring, 2002). Springs are, more or less, sealed from the atmosphere, which results in different dynamics of soluble gases compared to open systems (Snoejink & Jenkins, 1980; Döring, 2002). For example, dissolved oxygen concentrations often are low, although saturation may react rapidly in turbulent spring brooks (Van der Kamp, 1995; Döring, 2002).

2.1.3.1. Physical and chemical water parameters:

Temperature

According to McCaffrey (2013) temperature of a waterway is significant because it affects the amount of dissolved oxygen in the water. The amount of oxygen that will dissolve in water increases as temperature decreases. Water at 0^{o}C will hold up to 14.6 mg of oxygen per liter, while at 30^{o}C it will hold only up to 7.6 mg/L. Temperature also affects the rate of photosynthesis of plants, the metabolic rate of aquatic animals, rates of development, timing and success of reproduction, mobility, migration patterns and the sensitivity of organisms to toxins, parasites and disease. Life cycles of aquatic organisms are often related to changes in temperature (McCaffrey, 2013). Water temperatures can fluctuate seasonally, daily, and even hourly, especially in smaller sized streams. Spring discharges and overhanging canopy of stream vegetation provides shade and helps buffer the effects of temperature changes (Anonymous, 1996). Alfaro and Wallace (1994) have been recognized five classes for water temperature in springs based on a comparison of springs water temperature with the mean annual air temperature: cold, normal, warm or hot, and superthermal springs (Springer et al., 2008). Cold water springs are, by convention, <12.2^{o}C cooler than the mean annual ambient temperature. Spring waters within 12.2ºC of the mean ambient temperature may be (but are not necessarily) responding to ambient atmospheric temperatures. This is to be expected in springs that emerge from shallow aquifers and these may have temperatures that vary seasonally with air temperature (Springer et al., 2008). Springs with warm (>12.2ºC above the mean ambient air temperature, but <37.8ºC) and thermal water (>37.8ºC) are connected to either very large aquifers with long flow paths, or to geothermal sources of heat (Springer et al., 2008). Temperature affects the concentration of dissolved oxygen in a water body. Oxygen is more easily dissolved in cold water (Anonymous, 2001). According to Ward (1992), the temperature measured in a spring usually is equal to the average annual air temperature in the same region. However, such conditions may not be present in all systems (Döring, 2002 ex. Smith, 1985). Increasing distances from the source results in increasing annual and diurnal variability in response to the ambient air temperature and solar radiation (Döring, 2002 ex. Resch, 1983), whereby closed canopies and steep valleys reduce the effect of radiation on the water temperature (Döring, 2002). For example, Resh (1983) recorded a diurnal temperature range of 20 °C, 19 m downstream of the source of a Californian spring. Increasing discharge lowers the daily fluctuation and extends the stabilizing influence to the spring brook (Döring, 2002 ex. Smith & Lavis, 1975).

Bicarbonate, Calcium, and pH

pH is a term used universally to express the intensity of the acid or alkaline condition of a solution. pH is considered as an important ecological factor and provides an important piece factor and piece of information on many types of geochemical equilibrium or solubility calculation (Shyamala et al., 2008). Changes in pH can change the aspects of water chemistry. For example, as pH increases, smaller amounts of ammonia are needed to reach a level that is toxic to fish. As pH decreases, the concentration of metal may increase because higher acidity increases their ability to be dissolved from sediments into the water (Anonymous, 1996).

As rainfall passes through the soil layer it becomes increasingly acidic (low pH) as a result of its reaction with the carbon dioxide (CO_2) to form carbonic acid ((H_2CO_3) and by addition of natural organic acids (humic acid, etc.). Carbonic acid reacts with limestone ($CaCO_3$) to produce dissolved calcium (Ca^{2+}) and bicarbonate (HCO_3^-)(Champion & Starks, 2001). As the limestone is dissolved by carbonic acid, pH increases because carbonic acid is consumed and calcium and bicarbonate are produced (Champion & Starks, 2001). In a limestone aquifer, the concentrations of calcium and bicarbonate ions and the pH of groundwater are important indicators of chemical maturity or the extent of chemical equilibrium with the aquifer rock materials. Water that has been in contact with limestone for a relatively short length of time should have low concentrations of these ions and a relatively low pH, while water that has been in the flow system for a long period of time should typically have higher concentrations and a higher pH (Champion & Starks, 2001).

Groundwater discharging from the springs is generally dominated by calcium-bicarbonate, except along the coast where the coastal transition zone becomes an important influence on water quality. In addition, the pH of spring water typically varies between 7 and 8, indicating that the groundwater is at or near chemical equilibrium with limestone in the aquifer (Champion & Starks, 2001).

Dissolved Oxygen (DO)

DO is one of the most important parameters. Its correlation with water body gives direct and indirect information e.g. bacterial activity, photosynthesis, availability of nutrients, stratification etc. (Premlata, 2009). In the progress of summer, dissolved oxygen decreased due to increase in temperature and also due to increased microbial activity (Moss,1972; Morrissette & Mavinic, 1978; Kataria et al., 1996, Patil et al., 2012). The high DO in summer is due to increase in temperature and duration of bright sunlight has to influence on the % of soluble gases (O_2 and CO_2). During summer the long days and intense sunlight seem to accelerate photosynthesis by phytoplankton, utilizing CO_2 and giving off oxygen. This possibly accounts for the greater qualities of O_2 recorded during summer (Krishnamurthy, 1990). Normally, DO levels in natural streams are fairly high unless there are large quantities of organic debris, sewage effluents (discharges of oxygen demanding substances), and high water temperatures (Marsalek et al., 2005). The amount of oxygen in the water, to a degree, shows its overall health. That is, if oxygen levels are high, one can presume that pollution levels in the water are low. Conversely, if oxygen levels are low, one can presume there is a high oxygen demand and that the body of water is not of optimal health (McCaffrey, 2013).

The concentration of dissolved oxygen in a stream is affected by many factors (Anonymous, 1996):

- Temperature: Oxygen is more easily dissolved in cold water.
- Flow: Oxygen concentrations vary with the volume and velocity of water flowing in a stream. Faster flowing white water areas tend to be more oxygen-rich because more oxygen enters the water from the atmosphere in those areas than in slower, stagnant areas.
- Aquatic Plants: The presence of aquatic plants in a stream affects the dissolved oxygen concentration. Green plants release oxygen into the water during photosynthesis. Photosynthesis occurs during the day when the sun is out and ceases at night. Thus in streams with significant populations of algae and other aquatic plants, the dissolved oxygen concentration may fluctuate daily, reaching its highest levels in the late afternoon and may drop significantly by early morning.
- Altitude: Oxygen in more easily dissolved into water at low altitudes that at high altitudes.
- Dissolved or suspended solids: Oxygen is also more easily dissolved into water with low levels of dissolved or suspended solids.

Biochemical Oxygen Demand (BOD)

BOD is a measure of organic material contamination in water, specified in mg/L. BOD is the amount of dissolved oxygen required for the biochemical decomposition of organic compounds and the oxidation of certain inorganic materials (e.g., iron, sulfites). Typically the test for BOD is conducted over a five-day period (Patil et al., 2012).

Chemical Oxygen Demand (COD)

COD is another measure of organic material contamination in water specified in mg/L. COD is the amount of dissolved oxygen required to cause chemical oxidation of the organic material in water. Both BOD and COD are key indicators of the environmental health of a surface water supply (Patil et al., 2012).

Electrical conductivity (EC)

Electrical conductivity (EC) is a measure of water capacity to convey electric current. It signifies the amount of total dissolved salts (Dahiya & Kaur, 1999; Prasad et al., 2014). Conductivity shows significant correlation with ten parameters such as temperature, pH value, alkalinity, total hardness, calcium, total solids, total dissolved solids, chemical oxygen demand and chloride and iron concentration of water (Prasad et al., 2014).

Total Dissolved Solids (TDS)

Total Dissolved Solids (TDS) is a measure of all the chemical constituents dissolved in the groundwater. TDS is often used to stimate the relative residence time of ground water in the aquifer. TDS typically increases as the length of ground-water flow paths increase, and in coastal areas is often used to determine the influence of salt water on water quality (Champion & Starks, 2001). This is especially important in coastal springs where the coastal transition zone strongly influences ground-water quality (e.g., King's Bay Springs). In coastal springs that are tidally influenced, TDS may vary several thousand milligrams per liter over a given tidal cycle (Champion & Starks, 2001).

Water containing more than 500 mg/l of TDS is not considered desirable for drinking water supplies, but in unavoidable cases 1500 mg/l is also allowed (Shrinivasa & Venkateswaralu, 2000), highly mineralized water may be used where better quality water is not available (Jain, 2002).

Total Hardness (TH)

Hardness is the property of water, which prevents the lather formation with soap and increases the boiling points of water (Patil & Patil, 2010). The hardness of water mainly depends upon the amount of calcium or magnesium salts or both. According to some classifications, water with hardness up to 75 mg/l is classified as soft, 76 – 150 mg/l is moderately soft, 151 – 300 mg/l as hard and more than 300 mg/l as very hard (Saravanakumar & Ranjith Kumar, 2011).

Total alkalinity (TA)

It is composed primarily of carbonate (CO_3^{2-}) and bicarbonate (HCO_3^{-}), alkalinity acts as a stabilizer for pH. Alkalinity, pH and hardness affect the toxicity of many substances in the water (Patil et al., 2012).

Nutrients (nitrogen and phosphorus)

Nutrients are important constituents affecting the productivity of aquatic systems (Zalewski et al., 2004; Marsalek et al., 2005). Both N and P may originate from natural sources, but the main concern is caused by nutrients originating in sewage effluents, industrial discharges, and agricultural and urban runoff. Increased nutrient loadings may cause nutrient enrichment of surface waters and lake eutrophication, characterized by an overall increase of aquatic macrophytes and algal biomass, and changes in the composition of the algal community from one-celled diatoms to filamentous green forms, followed by blue-green forms. Interest in N and P stems from their potential bioavailability and toxicity. For example, short-lived nitrite nitrogen is toxic to salmonids at 0.1 to 0.9 mg of NO_2^-, and ammonia is toxic to salmonids at concentrations as low as 0.080 mg, depending on pH and DO (Spence et al. 1996).

Ammonium (NH_4^-) in ground water can be derived from a number of sources, including animal waste products, decay of complex organic molecules, and the use of inorganic fertilizers. It can only persist in reducing environments and/or in the immediate vicinity of a source. Ammonium is strongly absorbed to clays and organic materials disseminated within an aquifer (Champion & Starks, 2001 ex. Upchurch, 1992). Low ammonium concentrations reflect the conversion of the ammonium to nitrate in an oxidizing ground-water environment prior to reaching the springs. Low ammonium concentrations also indicate that any nitrogen sources contributing nitrogen to the ground-water system are not in the immediate vicinity of the springs (Champion & Starks, 2001). Nitrite (NO_2^-) is very unstable in the ground-water environment. Nitrite is the primary step in the oxidation of ammonium and other nitrogen compounds to nitrate. Because nitrite is chemically unstable, it can usually only persist near a source. A lack of significant nitrite concentrations in spring water indicates that the aquifer is an oxidizing environment. It also indicates that nitrogen sources contributing nitrite to the ground-water system are not in the immediate vicinity of the springs (Champion & Starks, 2001). Relatively common nitrate (NO_3^-), which is formed by oxidation of ammonia through nitrification, is essentially non-toxic to aquatic vertebrates and invertebrates. Nitrate is an important component in the cycling of nutrients (nitrogen and phosphorus) in plant and animal physiology (Champion & Starks, 2001). Nitrate typically act as a fertilizer in soils and surface-water bodies where it promotes growth of terrestrial and aquatic vegetation. However, high amounts of nitrates may lead to excessive growth and eventual eutrophication of surface-water bodies, especially those that are nitrogen limited (Champion & Starks, 2001). The likelihood of eutrophication, and the increasing amounts of nitrate seen in water bodies and aquifers across the United States, has alerted many to the dangers of nitrate in surface and ground water across the country. In fact, the United States Environmental Protection Agency – USEPA recently stated that nutrients (nitrogen and phosphorus) are the leading causes of water-quality impairment (Champion & Starks, 2001).

Phosphorus is an essential nutrient for living organisms and exists in water bodies as both dissolved and particulate species. It is generally the limiting nutrient for algal growth and, therefore, controls the primary productivity of a water body (Chapman & Kimstach, 1996 in UNESCO et al., 1996). Artificial increases in concentrations due to human activities are the principal cause of eutrophication. Phosphorus is rarely found in high concentrations in freshwaters as it is actively taken up by plants (Chapman & Kimstach, 1996 in UNESCO et al., 1996). As a result there can be considerable seasonal fluctuations in concentrations in surface waters. In most natural surface waters, phosphorus ranges from 0.005 to 0.020 mg/L PO_4-P. Concentrations as low as 0.001 mg/L PO_4-P may be found in some pristine waters and as high as 200 mg/L PO_4-P in some enclosed saline waters. Average groundwater levels are about 0.02 mg/L PO_4-P (Chapman & Kimstach, 1996 in UNESCO et al., 1996). As phosphorus is an essential component of the biological cycle in water bodies. High phosphorus concentrations in ground water indicate localized contamination by organic sources, such as human and animal wastes (Chapman & Kimstach, 1996 in UNESCO et al., 1996).

2.1.4. Geochemistry in springs

Among the physicochemical properties of water, its solvent action is geochemically of foremost importance. The solution processes are connected with electrolytic dissociation, whereby electrically charged ions are produced. The hydrogen-ion concentration of aqueous solutions is also of importance of geochemistry, as Atkins (1930) first pointed out, he gave the value for the pH of springs in calcareous regions 6.0 – 6.6 (Rankama & Sahama, 1968). The pH in soils is usually 2 – 12, but most soils are neutral in reaction. In peat soils the pH varies between 4.4 and 7.3 and in mineral soils between 4.8 and 8.2. The pH of soil regulates the metal uptake of plants by affecting the solubility of salts in soil solutions; but other factors also are effective as regulating agents. Dissolve carbon dioxide is important in regulating the pH of waters. Photosynthesis tends to increase the pH of water, owing to the consumption of the carbon dioxide. Limestone beds may regulate the pH of circulating pore solutions. Many deposits of zinc and other metal are the result of neutralizing action of limestone on acid hydrothermal solutions until the pH of precipitation of zinc sulfide is reached (Rankama & Sahama, 1968).

In the past, Rankama and Sahama (1968) stated that the composition and salinity of spring water depend on localon local conditions. Sulfates and carbonatesare the most important constituents of spring water, whereas chloride is is usually less important. The silica content may sometimes be rather high. Calcium is, quantitatively, the most important cation, and consequently th spring and well waters are hard. Usually the amount of carbonate exceeds that of sulfate, but, if much sulfides or sulfates are present in the percolated beds, the content of sulfate may be very high (Rankama & Sahama, 1968). According to Webb et al. (1998) the physical and chemical composition of spring water reflects not only the mineral composition of the various rock strata with which the water has been in contact, but also the various chemicals that percolate into ground water (Wetzel & Webb, 2007).

Springer et al. (2008) recommend that eight groups of geochemical variables be measured during springs inventories: major anions and cations (chloride, sulfate, carbonate, calcium, sodium, potassium), minor constituents (iron, borate, silica dioxide, carbonate/chloride, triple waters), pollution indicators (selenium, fecal colliform), useful tracers (stable isotopes, radioactive isotopes, rare-earth elements), alkalinity, total dissolved solids concentration and specific conductance, pH, and nutrient concentrations (nitrate and phosphate).

2.1.4.1. Geochemical fractions

In the case of metal chemistry, the metal species are very important for scientists to determine the behavior and toxicity of metals in a variety of chemical forms in the environment (Wijaya et al., 2016). The free metal ions, metal complexes dissolved in water and adsorbed on sediment surfaces, and metal species that have been co-precipitated in major metallic sediments (silica and alumina) are considered as part of the variety and complex forms found in sediment. The incorporation of metals into the sediment system also greatly depends on the form and range of the metal species, the charge on the metal entering the sediment, and the pH of the receiving environment (Wijaya et al., 2016). Metals may be present in soil in several different physicochemical phases, including sinks for soluble or exchangeable elements in the environment and amorphous material (Fe/Mn oxides) bound to organic matter, sulphides, or mineral fractions (residual) (Rauret et al., 1999; Zemberyov´a et al., 2006; Perez-Lopez et al., 2010; Nemanti et al., 2011 ex. Wali et al., 2014). Determining their fractionation is important because the mobility and bioavailability of metals are highly dependent on their specific chemical forms or 27ptimize of binding to each soil phase (Wali et al., 2014).

Fractionation is usually performed by using sequential extraction schemes (Rauret, 1998). The geochemical fractions obtained, when applying these schemes, are related to exchangeable metals, metals mainly bound to carbonates, metals released in reducible conditions such as those bound to hydrous oxides of Fe and Mn, metals bonded to oxidable components such as organic matter and sulphides, and residual fraction; which together form the total level concentrations (Rauret, 1998). Sequential extraction procedures provide more information on the origin, physicochemical availabilities, mobilization, and transport of metals in natural environments (Kierczak et al., 2008; Rao et al., 2010; Nemanti et al., 2011; Aiju et al., 2012 ex. Wali et al., 2014). The pH, redox potential, and organic matter can stimulate the mobilization and retention of trace metals in the natural environment (Tessier et al., 1979; Yang et al., 2009; Naji et al., 2010 ex. Wali et al., 2014). The European Community Bureau of Reference (1993) introduced a new three-step sequential extraction that has since been modified by Rauret et al. (1999). This BCR (Bureau Commune de Reference) sequential extraction method analyses different fractions of metals in the soil: acid-extractable (water soluble, exchangeable, and bound to carbonates), reducible (bound to Fe and Mn oxides), oxidizable (bound to sulphides and organic matter), and residual (Zemberyov´a et al., 2006; Pueyo et al., 2008; Saracoglu et al., 2009; Raži´c & Dogo, 2010; Wali et al., 2014).

2.1.5. Spring Biota

Spring invertebrate communities may be composed of groundwater, rhithral and terrestrial fauna and may include crenobionts (spring specialists) and crenophilic (showing maximum densities in springs) species depending on bioregional, climatic and environmental factors (Botosaneanu 1998; Barquín & Death, 2009). Therefore Dumnicka et al. (2007) affirmed the composition of fauna living in springs is influenced by hydrogeological conditions, their surroundings, zonal differences eucrenal–hypocrenal and also by disturbances, especially in the form of anthropopression (Dumnicka & Rozkowski, 2010). Within the spring, environmental characteristics change gradually downstream and the boundary of the spring area (eucrenal) has been established as the point where water temperature differs 2°C from water temperature at the source (Erman & Erman, 1995; Barquín & Death, 2009), or at 5 m from the springhead (Fumetti et al., 2006; Barquín & Death, 2009). Thus, invertebrate community composition will be highly influenced by spring type and proximity to the source (Barquín & Death, 2009). Light is a fundamental variable for benthic algae for photosynthesis, but also temperature, nutrients, grazers, substrate stability, substrate size and velocity (Rolland et al., 1997; Hill, 1996; Döring, 2002). Temperature affects biochemical reactions (DeNicola, 1996; Döring, 2002); low nutrient levels can stipulate growth limitation (Borchard, 1996; Döring, 2002); and unstable substrate condition can lead to physical damage and lowered light availability (Peterson, 1996; Döring, 2002).

Springs that represent extreme environments often support large mats of cyanobacteria, sulfide or iron oxidizing bacteria, or consortia of bacteria and archaea. Very often these define the nature of the venting fluids and form the visually dominant life form (Springer et al., 2008). For all lotic environments, it was confirmed that fauna diversity increases with greater substratum heterogeneity (Armitage et al., 1995). In springs, this problem has not been studied so frequently (Botosaneanu, 1998). Many springs represent a stable ecological environment, because their physical and chemical variables were kept constant all throughout the year (Barquín & Death, 2009 ex. Odum, 1971). However, they may also have a high degree of substratum heterogeneity, which results in high species richness. This has been discovered to occur mainly in large helocrenes and limnocrenes (Lindegaard et al., 1998). Various chemical factors have been identified to influence the specific spring fauna (Glazier, 1991; Williams et al., 1997; Orendt, 2000; Hahn, 2000; Von Fumetti, 2008). In springs, fauna-substratum relationships are considered to be complex because springs are highly diverse and some taxa have no specific substrate preferences (Zollhöfer, 1999; Döring, 2002). Diverse (taxonomy, life forms) algae assemblages reach highest abundances on pebble and cobble substratum (Cattaneo, et al., 1997; Rolland et al., 1997; Döring, 2002). Different chemical conditions may also effect the composition of algal biofilms (Allan, 1995; Döring, 2002).

Studies concerning factors influencing the occurrence of fauna have been carried out in European springs of various types (Thorup & Lindegaard, 1977; Tapia, 1996; Lindegaard et al., 1998). Community composition and diversity have also been compared but mostly among springs treated as a whole. Only sporadically were studies undertaken of fauna settlement in springs having different types of sediments. Several studies on the bottom fauna in various kinds of springs, situated on diverse bedrocks have been carried out (Fischer et al., 1998; Lindegaard et al., 1998; Sambugar, et al. 2006; Von Fumetti, 2008). Permanent springs establish specific habitat for aquatic fauna and flora due to the stability of water temperature, what results in the presence of oligostenothermic species. Due to small dimensions of many springs niches the influence of surrounding terrestrial hbitas on the availability of food (various kinds of organic matter) as well as on the composition of fauna may be distinct. As a consequence even in small springs invertebrate species having different ecological requirements could be found (Rozkowski & Dumnicka, 2010).

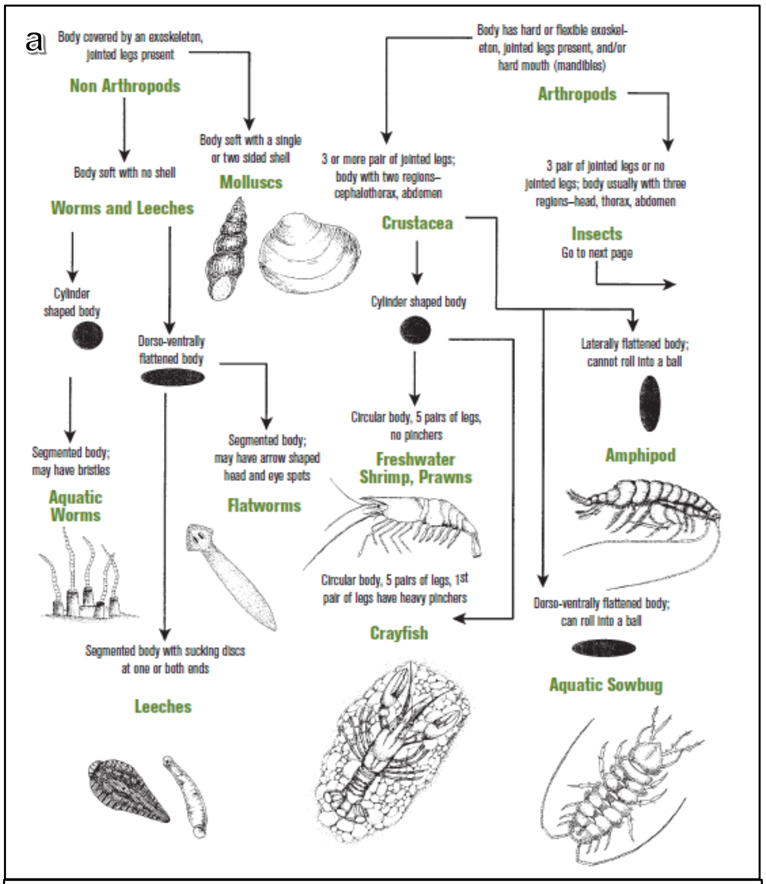
A research of Erman (2002) of long-term studies (over 20 years) of springs and spring invertebrates (Sierra Nevada, California, U.S.A.), showed the highly individualistic nature of springs even within the same stream basin. Spring invertebrate assemblages differed greatly from one spring to another, as did timing of insect emergence and abundance of species. Invertebrate species richness was greater in deeper, more permanent springs with high concentrations of dissolved ions, especially calcium (Erman, 2002). In this study, only five major groups of aquatic invertebrates were found in all 21 springs surveyed: nematodes, oligochaetes, water mites, caddisflies, and chironomids. The absence of flatworms and stoneflies in a spring indicated that a spring was not permanent over the long term (Erman, 2002).

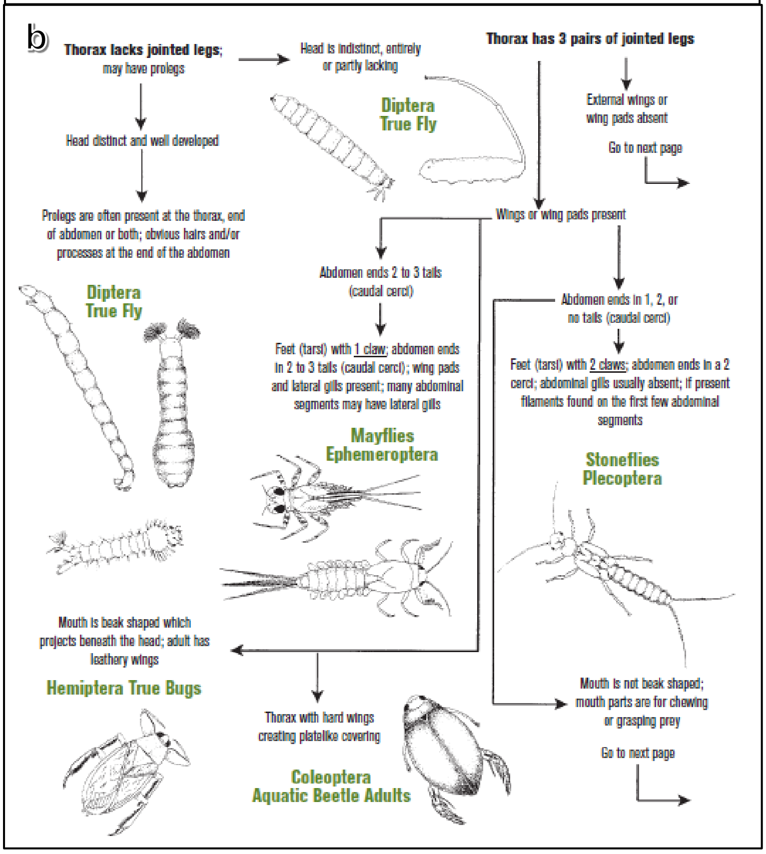
2.1.5.1. Benthic macroinvertebrates:

Benthic macroinvertebrates are organisms without backbones that inhabit the bottom substrates (Fig. 7) (for example, sediments, debris, logs, macrophytes, and filamentous algae) of their habitats, for at least part of their life cycle (Rosenberg & Resh, 1993). Benthic macroinvertebrates are visible to the naked eye and are retained by mesh sizes greater than or equal to 200 to 500 micrometers. Macroinvertebrates include insect larvae, annelids (leeches), oligochaetes (worms), crustaceans (crayfish and shrimp), mollusks (clams and mussels), and gastropods (snails).

Insect larvae tend to be the most abundant benthic macroinvertebrates in freshwater aquatic ecosystems (De Lange et al., 1994). As benthic macroinvertebrates tend to remain in their original habitat, they are affected by local changes in water quality. Some are capable of tolerating higher loads of pollution than others. Thus if the pollution is severe, or is moderate but sustained over time, the whole community structure may simplify in favor of tolerant species. Although the abundance of certain species may increase, the diversity and species richness decrease (De Lange et al., 1994). Along with their associated seeps and outflow brooks, springs provide a unique habitat for endemic species (organisms restricted to a localized area) of animals and plants because they usually provide a nearly constant physical and chemical environment (Wetzel & Webb, 2007). According to Wetzel and Webb (2007), past studies of springs generally focused on a selected taxonomic group, but provided minimal water quality information other than water temperature, dissolved oxygen, hydrogen ion concentration (as pH) and alkalinity. Aquatic macroinvertebrates of temperate, cold water areas are dominated by either a non-insectan community (Turbellaria, Annelida, Amphipoda, Isopoda, Gastropoda) or an aquatic insect community (Odonata, Ephemeroptera, Plecoptera, aquatic Diptera, Trichoptera, and aquatic Coleoptera). Faunal dominance by non-insect species appears in hard water limestone springs with a pH >7.0 and alkalinity above 25 mg/L (as $CaCO_3$). Aquatic insects appear to dominate the fauna in both acidic and soft water springs – those with a pH <7.0, and those with an alkalinity below 25 mg/L, respectively (Wetzel & Webb, 2007).

There are four feeding groups of macroinvertebrates: shredders, filter-collectors, grazers, and predators. **Shredders**, such as stoneflies (Plecoptera), feed on plant material and some animal material, which is generally dead, and break it into smaller particles through their feeding and digestive process. **Collectors**, such as caddisflies (Trichoptera) and blackflies (Diptera), feed on fine particle organic material which they filter from the water. **Grazers**, such as snails and beetles, feed on algae and other plant material living on rocks and on plant surfaces. **Predators,** such as dobsonflies (Magaloptera) or dragonflies (Odonata), feed on other macroinvertebrates. Individual species may be generalists, and fit into more than one of these groups (as opposed to specialists) (De Lange et al., 1994).


a
Body covered by an exoskeleton, jointed legs present
Non Arthropods
Body soft with no shell
Worms and Leeches
Body soft with a single or two sided shell
Molluscs
Body has hard or flexible exoskeleton, jointed legs present, and/or hard mouth (mandibles)
Arthropods
3 or more pair of jointed legs; body with two regions–cephalothorax, abdomen
Crustacea
3 pair of jointed legs or no jointed legs; body usually with three regions–head, thorax, abdomen
Insects
Go to next page
Cylinder shaped body
Segmented body; may have bristles
Aquatic Worms
Dorso-ventrally flattened body
Segmented body; may have arrow shaped head and eye spots
Flatworms
Segmented body with sucking discs at one or both ends
Leeches
Cylinder shaped body
Circular body, 5 pairs of legs, no pinchers
Freshwater Shrimp, Prawns
Circular body, 5 pairs of legs, 1st pair of legs have heavy pinchers
Crayfish
Laterally flattened body; cannot roll into a ball
Amphipod
Dorso-ventrally flattened body; can roll into a ball
Aquatic Sowbug


b
Thorax lacks jointed legs; may have prolegs
Head is indistinct, entirely or partly lacking
Diptera True Fly
Thorax has 3 pairs of jointed legs
External wings or wing pads absent
Go to next page
Head distinct and well developed
Prolegs are often present at the thorax, end of abdomen or both; obvious hairs and/or processes at the end of the abdomen
Diptera True Fly
Wings or wing pads present
Abdomen ends 2 to 3 tails (caudal cerci)
Feet (tarsi) with 1 claw; abdomen ends in 2 to 3 tails (caudal cerci); wing pads and lateral gills present; many abdominal segments may have lateral gills
Mayflies Ephemeroptera
Abdomen ends in 1, 2, or no tails (caudal cerci)
Feet (tarsi) with 2 claws; abdomen ends in a 2 cerci; abdominal gills usually absent; if present filaments found on the first few abdominal segments
Stoneflies Plecoptera
Mouth is beak shaped which projects beneath the head; adult has leathery wings
Hemiptera True Bugs
Thorax with hard wings creating platelike covering
Coleoptera Aquatic Beetle Adults
Mouth is not beak shaped; mouth parts are for chewing or grasping prey
Go to next page

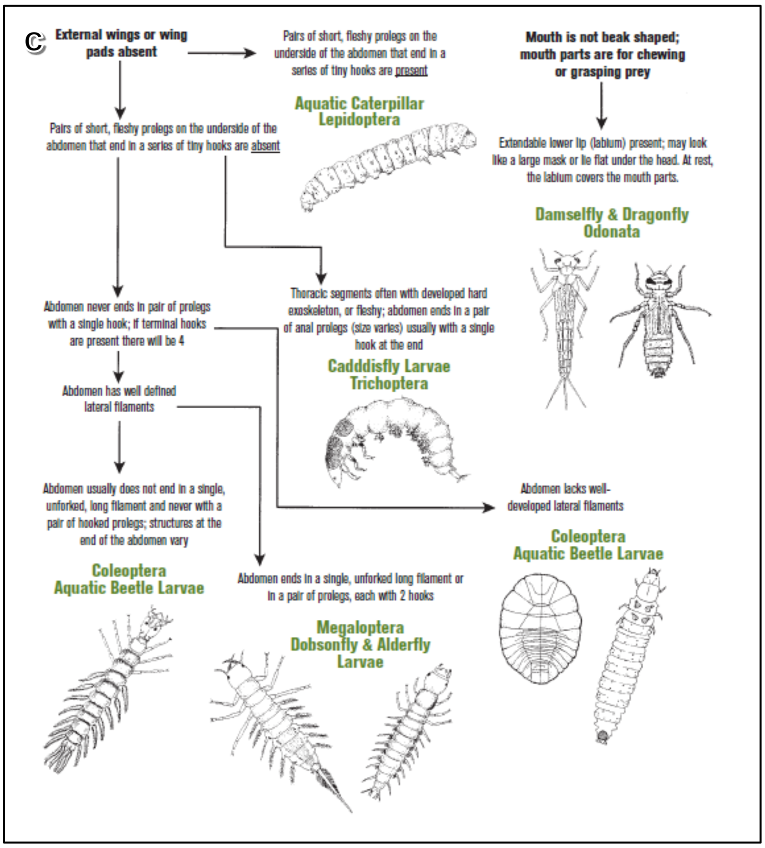


Fig. 7. Macroinvertebrates (**a** common freshwater macroinvertebrates; **b** and **c** aquatic Insect Orders of freshwater macroinvertebrates). Source: Texas commission on Environmental Quality, URL: http://www.eelriverrecovery.org/documents/Ch_6_Freshwater_Macroinvertebrates.pdf

2.2. TOXIC METALS

Toxic metals are a widespread problem in aquatic environments. Under natural conditions they are resistant to decomposition and can be accumulated in microorganisms, aquatic flora and fauna which, in turn, may enter into the terrestrial food chains (including human) resulting in further contamination of biota (Milošković et al. 2013). Toxic metals can be present in industrial, municipal and urban runoff, and by definition they are harmful to humans and aquatic biota (Okoro et al., 2012). Increased urbanization and industrialization have increased the levels of metal contents, especially in water ways. Mercury, lead, arsenic, cadmium, selenium, copper, zinc, nickel and chromium, however, should be given particular attention in terms of water pollution and discharge effects. Toxicity levels depend on the type of metals, its biological role, and the type of organisms that are exposed to it (Akan et al., 2010 ex. Okoro et al., 2012). There are various sources of metals; some originate from anthropogenic activities like industry, mining, draining of sewerage, urban drainage, dumping of hospital wastes and recreational activities. Conversely, metals also occur in small amounts naturally and may enter into the aquatic system through leaching of rocks, airborne dust, forest fires and vegetation (Ruqia Nazir et al., 2015). Major sources of metals in aquatic systems are the rock weathering and anthropogenic activities including industrial wastewater, drainage of land, atmospheric inputs, soil erosion, biological activities and urban wastes (Díaz de Alba et al., 2011; Carman et al., 2007).

The toxicity of metals in water depends on the degree of oxidation of a given metal ion together with the forms in which it occurs (Chapman & Kimstach, 1996 in UNESCO et al., 1996). As a rule, the ionic form of a metal is the most toxic form. However, the toxicity is reduced if the ions are bound into complexes with, for example, natural organic matter such as fulvic and humic acids. Under certain conditions, metallo-organic, low-molecular compounds formed in natural waters exhibit toxicities greater than the uncombined forms. An example is the highly toxic alkylderivatives of mercury (e.g. methylmercury) formed from elemental mercury by aquatic micro-organisms. Metals in natural waters can exist in truly dissolved, colloidal and suspended forms. The proportion of these forms varies for different metals and for different water bodies. Consequently, the toxicity and sedimentation potential of metals change, depending on their forms (Chapman & Kimstach, 1996 in UNESCO et al., 1996).

The effects of metals in water and wastewater range from beneficial through troublesome to dangerously toxic. Some metals are essential, others may adversely affect water consumers, wastewater treatment systems, and receiving waters. Some metals may be either beneficial or toxic, depending on concentration (Apha, 1995). This study focused on the concentration of dominant metals (Cu, Pb, Cd and Zn). While both Zn and Cu are bio-essential elements, they are potentially toxic at elevated concentrations (Fatoki, 1996). Lead, which is not considered to have any known biological function, can cause toxic effects and severe poisoning (Dao et al., 2012 ex. George & Goyer, 1978,).

Zinc: In slightly basic, anoxic marsh sediment environments, zinc is effectively immobilized and not bioavailable (Gambrell et al., 1991). Substantial amounts of zinc are released to solution if this sediment is oxidized or exposed to an acidic environment. Very high abundances of soluble zinc are present under well oxidized conditions and at pH 5 to 6.5, whereas low abundances of soluble zinc are present at pH 8 under all redox conditions and at pH 5 to 6.5 under moderately and strongly reducing conditions (Gambrell et al., 1991). In polluted river environment, most zinc is scavenged by non-detrital carbonate minerals, organic matter and oxide minerals and is less mobile than cadmium (and perhaps less mobile than lead) (Prusty et al., 1994). Elevated chloride contents decrease adsorption of zinc on sediment (Bourg, 1988).

Copper: Copper is an essential metal for most organisms (Clarkson et al., 1991) since the ions play an important role in their cellular metabolism (Karan et al., 1998). Beyond certain threshold levels (Prato et al., 2013) copper ions are extremely toxic for aquatic organisms and pose a threat to many aquatic organisms when available in excess (De Martinez et al., 2011). Copper ions can enter freshwater systems for example by runoff caused by strong rainfalls. In vineyards where fungicides containing copper ions are commonly applied (e.g. Ruyters et al. 2013), pollution of nearby springs is likely (Rychla et al., 2015). In most other situations lead is less mobile than copper. Elevated chloride contents decrease adsorption of copper on sediment, due to chloride complexation, which results in greater solubility and mobility (Bourg, 1988; Gambrell et al., 1991). In systems with high total copper contents, precipitation of malachite controls dissolved copper contents at low pH (Bourg, 1988; Salomons, 1995). Sometimes, elemental substitution is more complex; for example, copper toxicity is related to low abundances of zinc, iron, molybdenum, and (or) sulfate (Chaney, 1988).

Lead: The main sources of lead in the aquatic environment are leaded gasoline and mining (Prosi, 1989). Leaded gasoline results in introduction of organometallic lead compounds, which eventually reach surface water, into the atmosphere. Mining releases inorganic lead compounds. Both organic and inorganic forms of lead pose serious health risks to all forms of life (Ewers & Schlipköter, 1990). Inorganic lead compounds (sulfide, carbonate, and sulfate minerals) are commonly abundant in sediment but have low solubilities in natural water. Naturally-occurring lead in mineral deposits is not very mobile under normal environmental conditions, but becomes slightly more soluble under moderately acidic conditions. Soluble lead is little affected by redox potential (Gambrell et al., 1991). Lead is tightly bound under strongly reducing conditions by sulfide mineral precipitation and complexion with insoluble organic matter, and is very effectively immobilized by precipitated iron oxide minerals under well-oxidized conditions (Gambrell et al., 1991). In the aquatic environment, total dissolved lead abundances in water and pore water control primary uptake by organisms. Lead bioaccumulation is primarily dependent on the amount of active lead compounds (predominantly aqueous species) in the environment and the capacity of animal species to store lead (Prosi, 1989). Particulate lead may contribute to bioaccumulation in organisms. Studies of bottom dwelling organisms suggests that iron-rich sediment inhibits lead bioavailability (Luoma, 1989). In a polluted river environment, lead is most efficiently scavenged by non-detrital carbonate and iron-manganese oxide minerals and is less mobile than cadmium (Prusty et al., 1994). Pb generally tends to persist in the soil surface layers, although a significant quantity of Pb bound to the soluble part of the soil organic matter can be transported with percolates through the soil profile (Rieuwerts et al. 1998; Finžgar et al., 2007). Precipitation and complexation reactions of both Pb and Zn predominate at soil pH 6–7 (Rieuwerts et al. 1998; Finžgar et al., 2007).

Cadmium: cadmium (Cd) is considered as one of the most toxic heavy metals11 and an environmental pollutant toxic to a number of tissues (Klaassen et al., 1999). The persistence and ubiquitous nature of Cd is coupled with their tendency to accumulate in organisms ultimately produce toxic reactions in aquatic biota. Cd is highly toxic to some aquatic life. The important releases of Cd to the biosphere can be discussed as natural and anthropogenic activities. Anthropogenic releases are mainly from the mobilization of Cd impurities in raw materials (e.g., phosphate minerals, fossil fuels) and releases by manufacturing, use, disposal, recycling, reclamation or incineration of products intentionally (Unep, 2010). When Cd is introduced to freshwaters, the great bulk of the metal precipitates and resides in the bottom of sediments. Thus, sediment may be a significant source for Cd emitted to the aquatic environment (Lane & Morel, 2000). The effects of Cd on aquatic organisms can be directly or indirectly lethal and can impact populations and ecosystems as well as individuals. As a persistent environmental pollutant, Cd can alter trophic levels for centuries and freshwater organisms (Sorensen, 1991).

2.2.1. Level of the anthropogenic pollution by toxic metals in aquatic environment

Metals are naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water. In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by these metals (Bradl, 2002). Toxic chemicals can enter waters through direct discharge from industry or by improper disposal of industrial, mining, farm, and household wastes. In sufficient quantities, toxic substances, such as cleaning solvents, pesticides, and certain metals, can cause sickness, genetic disorders, and even kill organisms (Vandas et al., 2002). The over application of pesticides can result in the excess entering waters through runoff to surface water and infiltration into groundwaters. Even extremely low concentrations of some chemicals are hazardous to humans and aquatic life. Toxic substances also can affect an organism’s growth, metabolism, reproduction, or behavior. The potential dangers of many toxic substances are only now being recognized (Vandas et al., 2002). It has been reported that metals such as cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) are essential nutrients that are required for various biochemical and physiological functions. Inadequate supply of these micro-nutrients results in a variety of deficiency diseases or syndromes (Who et al., 1996). Their toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals. Because of their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance (Tchounwou et al., 2014). Pollutants enter aquatic environments and exert adverse effects on biota at the cellular, organism, population, and community levels, eventually altering the functioning of an aquatic ecosystem as a whole. Pollutants entering freshwaters and the oceans remain partly in solution and are partly adsorbed onto the surface of sedimentary matter (Inyang & Daniels, 2009).

The new generation of empirically based sediment quality guidelines (SQGs) was developed relying on field sediment chemistry paired with field or laboratory biological effects data. They are often based on frequency distributions and account for the impact of all chemicals present, but they do not establish cause and effect. These approaches have also been shown to be useful and predictive of biological effects in many (but not all) marine and freshwater systems (Long et al., 2000; MacDonald et al. 2000). Several empirical approaches have been published. These are based on the 37ptimized37ed37 of benthic macroinvertebrate effects and total sediment concentrations. They differ in the way they determine the threshold effects, but many are quite similar. These approaches include the effects range approach (Long & Morgan 1991; Ingersoll et al. 1996; Burton, 2002), effects level approach (Ingersoll et al. 1996; Burton, 2002), apparent effects threshold approach (Cubbage et al. 1997; Burton, 2002), and screening level concentration approach (Persaud et al. 1993; Burton, 2002). These approaches generally set two threshold levels, one below which effects rarely occur [e.g., the lowest effect level (LEL), threshold effect level (TEL), effects range low (ERL), minimal effect threshold (MET), and threshold effect concentration (TEC)], and one above which effects are likely to occur [e.g., the severe effect level (SEL), probable effects level (PEL), effect range median (ERM), toxic effect threshold (TET), and probable effect concentration (PEC)] (Burton, 2002).

MacDonald et al. (2000) developed “consensus” guidelines for total polychlorinated biphenyls (PCBs) using an approach similar to that of Swartz and then SQGs for freshwater sediments for 28 compounds, including metals, PAHs, PCBs, and pesticides. It is also useful to determine the magnitude of exceedances of multiple SQGs by using a hazard quotient approach (Long & MacDonald, 1998; Burton, 2002). In this approach, the concentration of each chemical is divided by the SQG. MacDonald et al. (2000) found that a mean PEC quotient of 0.5 is a useful threshold for determining whether or not sediment samples are toxic (Long & MacDonald, 1998; Burton, 2002).

Table 1 Threshold effect and Extreme effect sediment quality guidelines for metals (mg/kg) (Burton, 2002):

SQG	Cu	Pb	Zn	Cd
TEC	31.6	35.8	121	0.99
PEC	149	128	459	4.98

SQG, Sediment quality guideline
TEC, Threshold Effect Concentration
PEC, Persistance Effect Concentration
Source: MacDonald, et al., 2000.

2.2.2. Accumulation and uptake

The primary mechanism for toxicity to organisms that live in the water column is by absorption or uptake across the gills (U.S. EPA, 1996). Uptake of contaminants in the aquatic environment can occur via a number of different pathways (Bianchini & Bowles, 2002; Eggleton & Thomas, 2004; Marsden & Rainbow, 2004, Ward, 2013). These pathways include the uptake of metals via active transport across a cell membrane and passive diffusion for dissolved phase metal, and ingestion of food and sediment with associated particulate phase metals (Eggleton & Thomas, 2004; Campbell et al., 2006; Ward, 2013). In the case of sediment dwelling (benthic) organisms, metal accumulation may be the result of the combined exposure to dissolved (pore water, overlying water) or particulate (sediment ingestion, food) metals (Simpson et al., 2005; Simpson & King, 2005; Ward, 2013). The metals include copper (Cu), iron (Fe), cadmium (Cd), zinc (Zn), mercury (Hg), and lead (Pb) are the most toxic to aquatic organisms. Some water quality characteristics which affect metal toxicity include temperature, pH, hardness, alkalinity, suspended solids, redox potential and dissolved organic carbon. Metals can bind to many organic and inorganic compounds which reduce the toxicity of the metal (Anonymous, 1996).

The phases considered relevant in metals adsorption in sediments are oxides, sulphides and organic matter (Rauret, 1998). Dirilgen (2000) says that in natural systems, metals are not always freely available for uptake; a proportion may be bound in dissolved complexes. Thus, alterations of the physiochemical conditions of the environment (pH, redox potential, organic and inorganic ligands, T, etc.) can strongly influence the relative proportions of the metal ions that can be taken up.

Estimation of the total metal concentrations in sediments is not suitable to determine their mobility (Tüzen, 2003). Several mechanisms contribute to metal remobilization from contaminated sediment. First, sediment and associated contaminants may be resuspended by dredging, agitation from boat propellers, storms, floods, runoff, and activities of bottom-dwelling creatures (Lee & Jones-Lee, 1993; Horowitz, 1991). Second, changes in physicochemical conditions, such as pH or dissolved oxygen, can dissolve sediment-bound metals and make them available to enter the food chain (Horowitz, 1991). Finally, some inorganic or inert constituents can degrade or react with other constituents to create more soluble forms with higher toxicity (e.g., mercury to methyl-mercury) (Horowitz, 1991).

The accumulation of metals in sediments from both natural and anthropogenic sources occurs in the same way (Idris et al., 2007), consequently making it difficult to identify and determine the origin of metals present in the sediments (Okoro et al., 2012). Moreover, the total concentration of metals often does not accurately represent their characteristics and toxicity. In order to overcome the above mentioned obstacles it is helpful to evaluate the individual fractions of the metals to fully understand their actual and potential environmental effects (Okoro et al., 2012 ex. Tessier et al., 1979). Single extractions are thus used generally to provide a rapid evaluation of the exchangeable metal fraction in soils and sediments (Quevauviller & Olazabal, 2002; Sahuquillo et al., 2003). According to Sakan et al. (2007), metals from different sources normally accumulate together, mostly in fine-grained fractions of sediments and for this reason, the determination of metal contributions usually requires compensation for the grain size and mineralogical effects on the variability of a metal in different sediment samples (i.e. normalization).

Gheorghe et al. (2017) affirmed the metal bioavailability controls their accumulation in aquatic organisms. The factors that control the bioavailability of metals are the following: the organism biology (metals assimilation efficiency, feeding strategies, size or age, reproductive stage); metal geochemistry (distribution in water – sediment, suspended matters, and metal speciation) (Roosa et al., 2016); physical and chemical factors (temperature, salinity, pH, ionic strength, concentration of dissolved organic carbon, total suspended solids) (Fu et al., 2014; Bonnail et al., 2016). The metals uptaken paths are through the permeable epidermis if metals are in dissolved forms or through the food ingestion if metals are in particulate forms. The ingestion uptake depends on factors (the presence of organic or inorganic complexes, pH, temperature, salinity, and redox conditions) plus the rate of feeding, intestinal transit time, and the digestion efficiency (Gheorghe et al., 2017 ex. Bryan et al., 1985). Many studies have shown that the free hydrated metallic ion is the most bioavailable form for Cu, Cd, Zn (Roesijadi & Robinson, 1994), and Pb (Wojtkowska et al.,2016), but some exceptions have been reported (Wang & Fisher, 1997). Thus, the importance of other chemical forms of dissolved metals and complexes formed with suitable organic ligands with low molecular weight should not be neglected (Gheorghe et al., 2017). It has been found that the presence of organic binders increases the bioavailability of Cd in mussels (Gheorghe et al., 2017). The organic compounds of metals could be more bioavailable than the ionic forms (Chapman et al., 1998). Other studies highlighted that bioavailability of metals in bivalve mollusks depends on sediment particle size due to their filter feeding behavior. If the particles were coated with bacterial extracellular polymers or fulvic acids, the Cd, Zn, and Ag bioavailability was significantly increased. Overall, the binding of metal decreased the bioavailability of metals from the sediment (Wang & Fisher, 1997; Rosado et al., 2016).

Pollutants can be uptaken by organism directly from the environment or through ingestion of particles [53], and the accumulation occurs when an organism absorbs toxic chemical with a rate faster than the chemical is metabolized. On the contrary, the bioconcentration refers to the chemical uptake from the water only, which could be assessed in the laboratory conditions (Gheorghe et al., 2017). It is considered that a high bioaccumulation potential does not necessarily imply a high potential for toxicity, and as a result, the toxic effects should be estimated separately. In addition, it was made a distinction between accumulation in a small concentrations range, which occurs due to physiological needs (e.g., Zn) and apparently uncontrolled accumulation (e.g., Cd) (Iordache, 2009).

Metal concentrations such as Fe, Mn, Cu, Cr, and Pb were not amplified in the food chain (benthic fauna-fish-birds), but they were amplified for Zn and Cd (Gheorghe et al., 2017). Concentrations of metals were greater at the end of the trophic chains, as follows: vegetation/detritus – terrestrial invertebrates phytophase/detritophage – terrestrial invertebrates' predators – amphibians (Cd, Cr, Pb, and Cu in case of detritus chain and Zn in case of vegetation). The transfer of metals from benthic invertebrates to omnivorous fish revealed concentration of Zn and Cu in the liver and Zn in muscle (Iordache, 2009).

2.2.3. Toxic metals in sediments and biota of aquatic environment

Toxic metals are a widespread problem in aquatic environments. In the past, water quality studies focused mostly on the detection of contaminants in the water column and ignored the fact that sediments may act as large sinks or reservoirs of contamination (Horowitz, 1991). Many past studies also failed to recognize that remobilization of metals from contaminated sediment can cause water quality problems (Carvalho & Schropp, 2002). Sediments act as sinks and sources of contaminants in aquatic systems because of their variable physical and chemical properties (Pekey et al, 2006). Investigations pertaining to the metals in sediments have enlarged in recent years (Passos et al., 2010; Nguyen et al., 2009). Sediments are ecologically important components of aquatic habitat. The sediments may represent a historical record of water quality conditions at a particular site, the extraction of sediments brings up the issue of chemical partitioning and bioavailability (Marsalek et al., 1999).

Sediments show a great capacity to accumulate contaminants, and most pollutants adsorbed on the sediments are not bioavailable (Nemati et al., 2011). However, some processes such as sediments resuspension, desorption, redox reactions or degradation of the sorptive substance may induce pollutants release into the water column. Therefore, sediments not only act as a reservoir but also the potential source of pollutants in the aquatic system (Nemati et al., 2011). They are responsible for transporting a significant proportion of many nutrients and contaminants. They also mediate their uptake, storage, release and transfer between environmental compartments (Mohamed et al., 2014). The metals pollution of sediments is of major concern because of their toxicity, persistence and bio-accumulative nature and may be transferred to the overlying water and thereby entering into the food chain (Nemati et al., 2011; Díaz de Alba et al., 2011). Weathering is not the only source of trace metals in sediments. Organic material also carries trace metals (e.g., Hg and Cd). Naturally and anthropogenically derived non-detrital metals can also become associated with fine-grained inorganic and organic material during transportation and deposition and add to the total metal concentration in sediments (Carvalho & Schropp, 2002). In addition to the naturally occurring metals, sediments may also contain metals from human activities. Unfortunately, the variability of the natural sediment metal concentrations sometimes makes it difficult to determine whether a measured metal concentration represents a natural or metal-enriched condition (Carvalho & Schropp, 2002). Carvalho and Schropp (2002) state that in spite of input and transport dissimilarities, both natural and anthropogenic metals tend to accumulate together in sediments. As a result, separating and quantifying natural and anthropogenic metal contents becomes difficult. In the absence of anthropogenic intrusions, trace metals in sediments are mainly associated with silicates and primary minerals and therefore have limited mobility. However, the metals introduced by human activities show greater mobility and are associated with other sediment phases, such as carbonates, oxides, hydroxides and sulfides (Passos et al., 2010).

Under natural conditions toxic metals are resistant to decomposition and can be accumulated in microorganisms, aquatic flora and fauna which, in turn, may enter into the terrestrial food chains (including human) resulting in further contamination of biota (Milošković et al., 2013).

Aquatic organisms are generally more sensitive to Cu and Zn in water than are humans. For instance, fishes and crustaceans were 10-100 times more sensitive to Cu than mammals (Forstner et al., 1979; Flemming & Trevors, 1989; Wright & Welbourn, 2002; Fu et al., 2016). With increasing emissions to water, and sensitivity of aquatic species, Cu and Zn in aquatic environments might pose greater potential risks to aquatic organisms (Fu et al., 2016). The bioavailability and toxicity of metals to aquatic organisms depend on the physical and chemical forms of the metal as well as several physicochemical parameters (Okoro et al., 2012). Metal concentrations in biota are governed by the bioconcentration process. These processes vary within species and depend on the season and the biota’s development stage, behavior, sex, and its history of contaminant exposure (Eggleton & Thomas, 2004). The elevated concentration of metals in aquatic biota may cause a decrease in biodiversity and potential toxicological risk to the human population, for people consuming organisms with a high metal content in their tissue (Komínková et al., 2015).

The effects on organisms' growth and development were triggered by the inhibition of enzymatic systems involved in protein synthesis and cell division (Gheorghe et al., 2017). The metal type modulates the bioaccumulation level and enzymatic systems vulnerability generating a multitude of effects, toxic or not (Köhler et al. 2007; Triebskorn et al. 2008). In order to understand the interaction mechanism between the toxic metals and the aquatic organisms and how organisms answer to metal contamination, more information on bioavailability is needed (Zhou et al., 2007).

III. Methodology:

3.1. Study area

The study site comprised 20 samplig sites locate in the Lužické mountains springs, Liberec (North part of the Czech Republic) (Fig 8). The suburbs of Liberec have a population of 150.000 inhabitants which makes it the fifth largest city. The region shares international borders with Germany and Poland. Liberec is a city with a significant rainfall. Average annual rainfall is about 604 mm. The temperature average is 7.3 °C. The driest month is February (30 mm of precipitation), most of the precipitation falls in July (averaging 78 mm). With an average of 16.7 °C, July is the warmest month and January is the coldest month, with temperatures averaging -2.8 °C. Due to geology fault, the sampling sites were named according to their locations in three different zones (B, D and E) in the North and South (Fig. 9). Description of sampling sites are showed in Table 2.

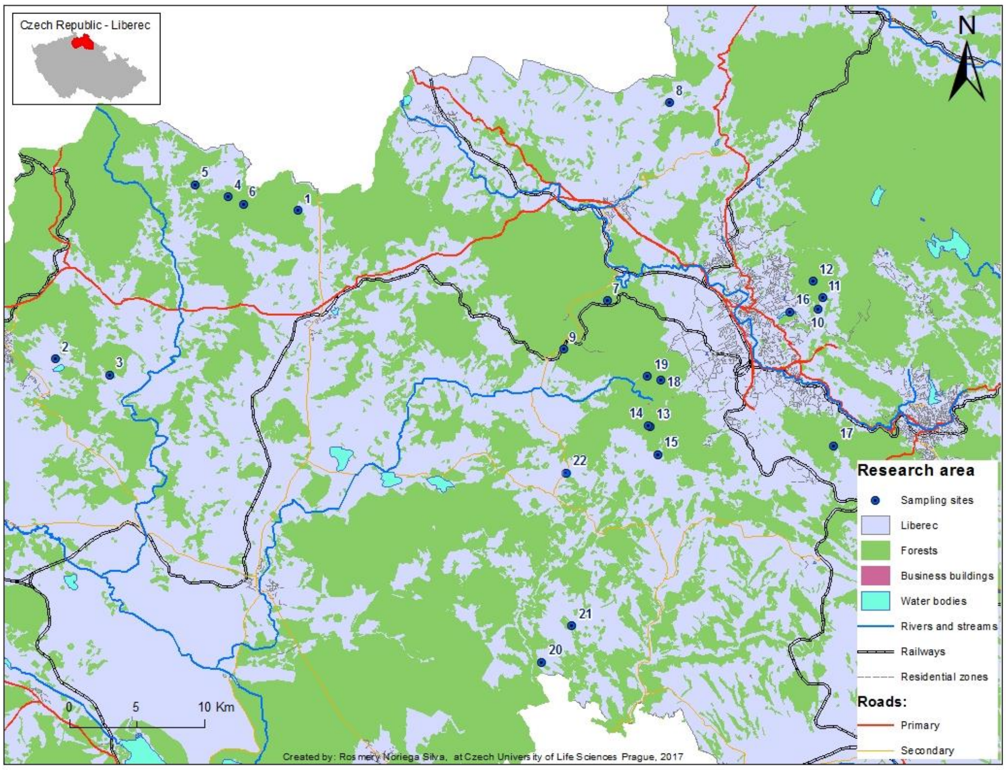


Fig. 8 Map of the study area with locations of the sampling sites.

Source: http://www.mapcruzin.com/free-czech-republic-arcgis-maps-shapefiles.htm

Table 2. Description of zones, localities, spring type (L-limnocrene, R-rheocrene, H-helocrene) and geology of sampling sites.

Zones	Springs					
	Site number	**Site name**	**Localities**	**Type**	**Geological description**	**Note**
BS (Zone B South)	1	**BS1007**	Petrovice - Kněžický potok	L	Cretaceous, quartz sandstones, locally glauconite-bearing, siltstones, claystones, marls	Dry - Unsampled
	2	**BS1016**	Radvanec	R	Cretaceous, sandstones, mostly quartzose	
	3	**BS1017**	Ortel	L	Cretaceous, sandstones, mostly quartzose	
	4	**BS1018**	Cesta ke Krompachu	L	Cretaceous, quartz sandstones, locally glauconite-bearing, siltstones, claystones, marls	
	5	**BS1019**	Krompach - dřevěný jímací obj.	R	Cretaceous, quartz sandstones, locally glauconite-bearing, siltstones, claystones, marls	
	6	**BS1027**	Heřmanický vodopád	R	Cretaceous, quartz sandstones	
DN (Zone D North)	7	**DN1010**	Kryštofovo údolí - vodárna	R	Cambrian (Ordovician), limestones, dolomites	
	8	**DN1012**	Vítkovský potok	H	Cambrian – ordovician, augen gneisses and granite gneisses	
	9	**DN1013**	Kryštofovo údolí	-	Carboniferous (Early Permian), Biotite-monzogranites, coarse grained, porphyric (Krkonoše type)	Unsampled
EN (Zone E North)	10	**EN1001**	Na Skřivanech	-	Carboniferous (Early Permian), Biotite-monzogranites, coarse grained, porphyric (Krkonoše type)	
	11	**EN1002**	Jizerská kompost	H	Carboniferous (Early Permian), Biotite-monzogranites, coarse grained, porphyric (Krkonoše type)	
	12	**EN1004**	Skautská studánka - Liberec	R	Carboniferous (Early Permian), Biotite-monzogranites, coarse grained, porphyric (Krkonoše type)	
	13	**EN1005**	Terasy Ještědu - Frantina studánka	R	Cambrian (Ordovician), sericite quartzites, locally metaconglomerates	
	14	**EN1006**	Pramen lesních panen	R	cambrian (Ordovician), sericite quartzites, locally metaconglomerates	
	15	**EN1011**	Jiříčkov	R	Devonian, limestones	
	16	**EN1015**	Harcov - Liberec	-	Carboniferous (Early Permian); Biotite-monzogranites, coarse grained, porphyric (Krkonoše type)	
	17	**EN1020**	Milíře	L	Carboniferous (Early Permian), muscovite-biotite monzogranites, medium-grained (Tanvald type)	
	18	**EN1021**	Mokřad Ještěd	H	Cambrian (ordovician), sericite quartzites, locally metaconglomerates	
	19	**EN1022**	Ostašovský potok - Ještěd	R	Cambrian (Ordovician), sericite quartzites, locally metaconglomerates	
ES (Zone E South)	20	**ES1008**	Vápno	L	Cretaceous, sandstones	
	21	**ES1009**	Lesnovek	R	Cretaceous, sandstones	
	22	**ES1014**	Janův důl	H	Cretaceous, quartz sandstones, locally glauconite-bearing, siltstones, claystones, marls)	

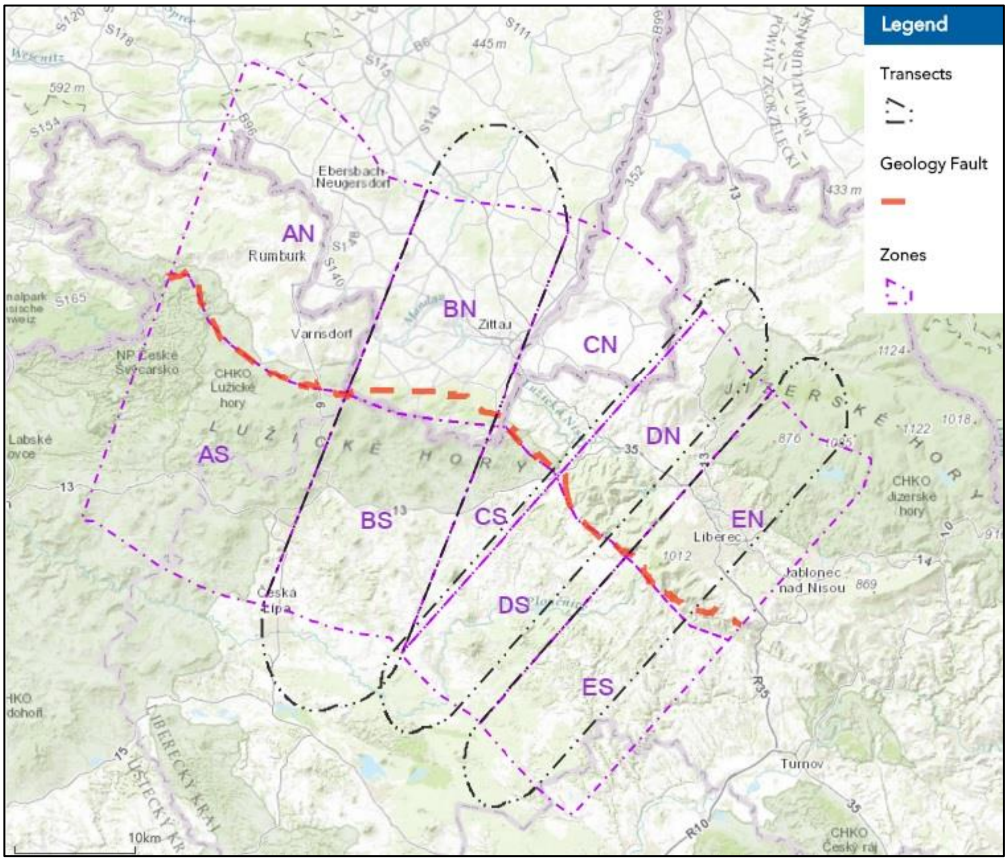


Fig. 9 Map of zones (BS, DN, EN, ES) and transections where sampling sites were located. Source:http://tuliberec.maps.arcgis.com/apps/webappviewer/index.html?id=12b41d82c18648a2b37bc8f502de95fe

The springs were classified in three types described by Thieneman (1929), limnocrene, rheocrene and helocrene. The distribution of springs to groups according to these types are as follows:

Limnocrene springs:

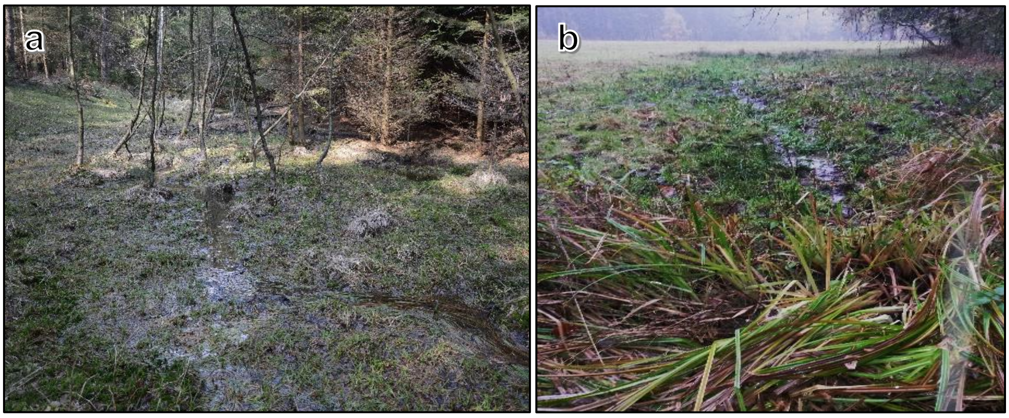


Fig. 10 (**a** BS1007, **b** BS1017, **c** BS1018, **d** EN1020, **e** ES1008)

Rheocrene springs:

Fig. 11 (**a** BS1016, **b** BS1019, **c** and **d** DN1010, **e** and **f** EN1004, **g** and **h** EN1005, **I** EN1006, **j** EN1011, **k** EN1022, **l** ES1009)

Helocrene springs:

Fig. 12 (**a** DN1012, **b** EN1002, **c** EN1021, **d** ES1014)

Three springs were not well identified, because their source are not natural, in case of EN1015 (Fig. 13) its source is clearly a pipe; EN1001 (Fig. 14) and DN1013 (Fig. 15) are also no natural source.

Fig. 13 EN1015

Fig. 14 EN1001

Fig. 15 DN1013

3.2. Sample collection and preservation

Both biotic and sediment samples were collected at the studied sites two times throughout the year 2016, in the period of June 21st – July 18th (Spring season) and in the period of October, 24th – 31st (Autumn season).

Water samples were collected at the studied sites only in autumn period and placed in clean polyethylene bottles (Fig. 16). For sediment sampling, approximately 0.5 kg of sediment was collected from the superficial layer (0-20 cm) by plastic scoop and placed in clean polyethylene containers and freeze-dried (at most sites, there was only a thin layer of sediment; hence, it was possible to sample sediment only to 5 cm depth) (Fig. 17).

Fig. 16 Water samples collection

Fig. 17 Sediment samples collection

Macroinvertebrates were collected by artificial substrate samplers (Fig. 18) (Taylor & Kovats, 1995) deployed on the bottom of the springs for a period of 1 month, sufficient to obtain an adequate sample for metal analyses (Komínková & Nábělková, 2007). Artificial samplers were made from sorted stone of grain size 0.5 – 2.5 cm. Organisms in the samplers and on their surface were collected in pouches, then they were identified by the family or species level (Fig. 19). The organisms were frozen and then freeze-dried, with the full content of their guts. This approach was applied to identify the amount of metals that can potentially be ingested by the predator, who consumes the entire prey. Hence, the predator can consume metals bound in biological tissue as well as metals present in sediment and water in the guts of the prey. All type of samples were placed in coolers and transported to the laboratory within several hours of sample collection (Marsalek et al., 1999).

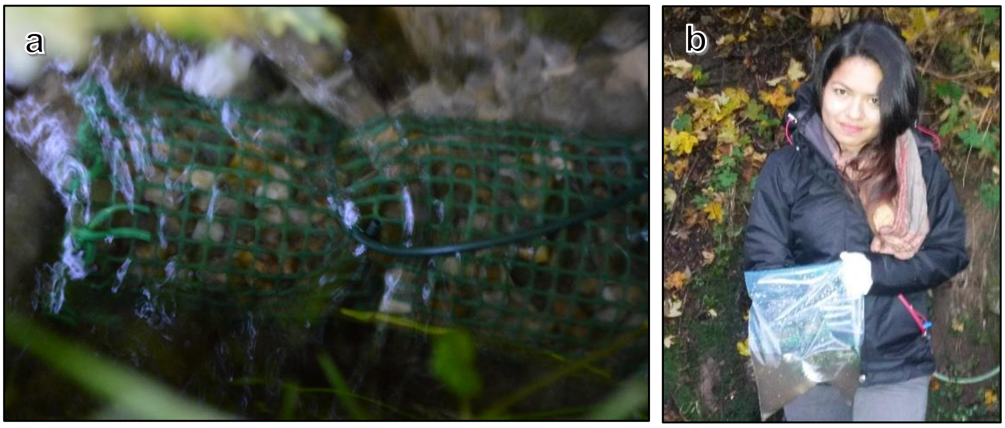


Fig. 18 (**a** an artificial substrate sampler in spring; **b** collection of samplers in pouches)

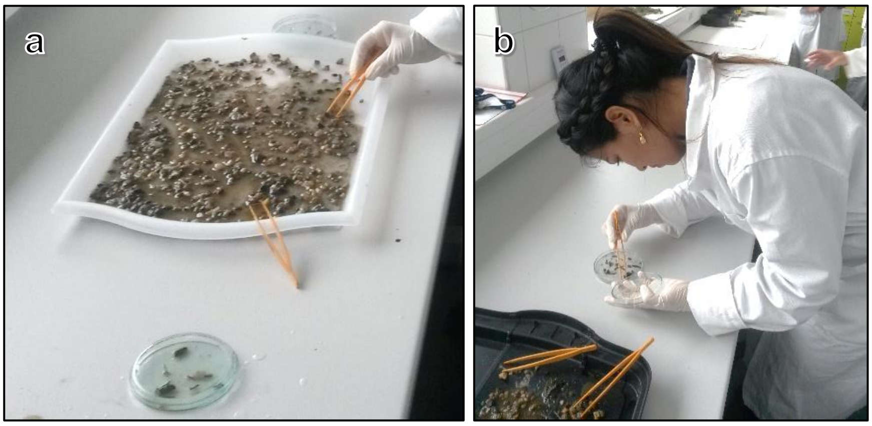


Fig. 19 (**a** and **b** identification and separation of biotic samples by type of species at laboratory)

3.3. Methods of chemical analyses

All analyses were carried out in the laboratory of the Department of Applied Ecology of the Czech University of Life Sciences Prague.

Basic parameters of physical – chemical quality of water of springs (Temperature, dissolve oxygen (DO), electric conductivity and pH) were determined in-situ using the handheld digital analyzer (HACH, HQd Field Case) (Fig. 20), Water samples were fixed by the addition of a small amount of HNO_3 and analyzed by an atomic absorption spectrometer with graphite furnace atomization (GFAAS) (Nábělková & Komínková, 2012).

Fig. 20 Physico – chemical water parameters (**a** and **b** measurement of water parameters using the handheld digital analyzer for pH, temperature, conductivity and DO)

Each sample of sediment was sieved through a sieving machine (FRITSCH, SPARTAN, Germany) with a frequency (Hz) 50/60, to separate into four fractions (Fig. 21): > 0.609 mm (Total); 0.609 mm – 0.206 mm; 0.206 mm – 0.061 mm and < 0.061 mm; for approximately 40 to 60 minutes to obtain at least 1 g of the last fraction.

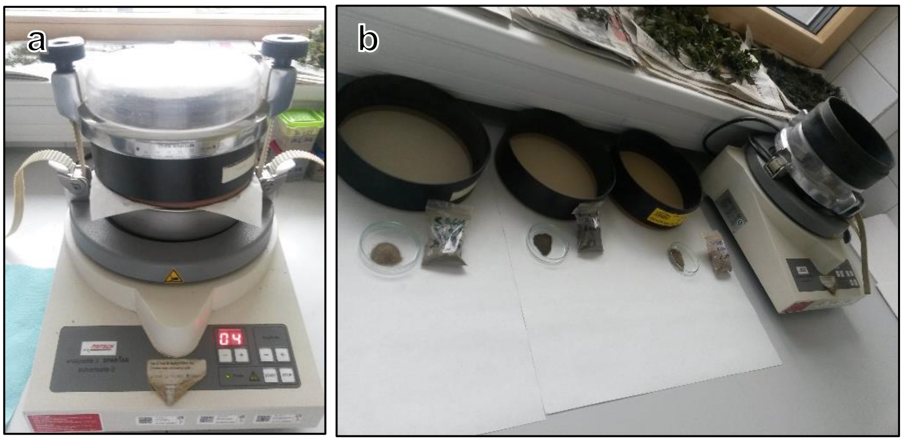


Fig. 21 Sieving of sediment samples (**a** sieving machine; **b** sediment fractions after sieving)

Microwave Digestion procedure

Sediment samples were digested following the US EPA 3052 microwave (MW) digestion method. A representative sample of 0.5 g up to 1.0 g of sediment fraction was placed in 9 ml nitric acid – HNO_3 (65%) and 1 ml hydrogen peroxide – H_2O_2 (30%). The sample and acid are placed in suitable inert Teflon microwave vessels (Fig. 22). The vessel was sealed and heated in a microwave oven (BERGHOF, MWS-2, Germany) at 180 °C for 30 min, (by program P2). After cooling, the digests were filtered (Fig. 23), adjusted to 50 ml with deionized Milli-Q water, and stored at 4 °C in polypropylene bottles until analysis. A procedure blank (reagents without any sample) was subjected to the same digestion procedure. This method provides information about the available fraction of the metal content (Bettiol et al. 2008).

The biotic samples were microwave digested. Digestion was performed in a microwave oven (BERGHOF, MWS-2, Germany) in two steps; first step, a maximum 0.2 g of crushed organisms was placed in 4 ml HNO_3 (67%) and 1 ml H_2O_2 (30%). The samples were put in vessels, then they were covered and left for 12 hrs in the laboratory. This step was to prevent a blustering reaction. The second step, add 5 mL of concentrated HNO_3 to the vessels of the previous step. The vessels are sealed and heated in a microwave oven (BERGHOF, MWS-2, Germany) at 180 °C for 30 min, with the microwave system P6 (by program P6). After cooling, the digests were filtered, adjusted to 10 ml with deionized Milli-Q water, and stored at 4 °C in polypropylene bottles until analysis. A procedure blank (reagents without any sample) was subjected to the same digestion procedure.

The accuracy of the analytical procedure for pseudototal metal concentration in sediment was checked using certified reference material by Metranal (sediment reference material QCM06, fine sediment; and QCM01, river sediment).

Fig. 22 Digestion procedure of sediment samples

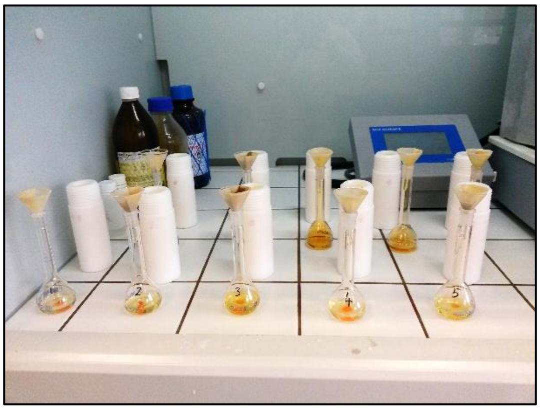


Fig. 23 Filtration of samples and adjusted to 50 ml with deionized Milli-Q water

BCR sequential extraction procedure

The binding behavior of metals to different geochemical fractions and the biological availability of metals bound to sediment were assessed by a sequential extraction procedure. Soil samples were analyzed by the modified BCR three-step sequential extraction procedure described by Nemati et al. (2011) and Davutluoglu et al. (2011). Sequential extraction involved three main steps and a fourth step as added by Rauret et al. (1999). In the first step, the exchangeable and soluble fractions were extracted. Extraction with acetic acid solution (40 mL of 0.11 mol L^{-1}) in a mechanical end-over-end shaker at 30 ±10 rpm at Digital orbital shaker (SHO-2D Model, 230 Volts, Korea) for 16 h. The extract was separated from the solid residue by centrifugation (MPW-251,18000rpm, Poland) (Fig. 24) at 6000 rpm for 10 min and collected in polyethylene bottles. The residue was washed by shaking for 15 min with 20 mL of deionized water and centrifuged for 10 min at 6000 rpm . The resulting supernatant was discarded.

In the second step, the reducible fraction was extracted. We added 40 mL of 0.5 mol/L hydroxyl ammonium chloride solution to the residue from the first step and re-suspended the mixture by mechanical shaking for 16 h at RT. The acidity level of this reagent was adjusted to pH 1.5 with HNO_3. The extract was separated, and the residue was washed in the same manner as described in the first step.

In the third step, the oxidizable fraction was extracted. We cautiously added 10 mL of 8.8 mol L^{-1} H_2O_2 solution to the residue from the second step and digested the mixture for 1 h at RT and 1 h at 85 ± 2 °C (Fig. 25). The volume was reduced to less than 3 mL. A second aliquot of 10 mL of H_2O_2 was added to the mixture, which was digested for 1 h at 85 ± 2 °C until the volume of the residue reached 2 to 3 mL (Fig. 26). The residue was mixed with 40 mL of 1 mol L^{-1} ammonium acetate solution, adjusted to pH 2 with HNO_3 and shaken for 16 h at RT. The extract was separated, and the residue was washed in the same manner as described in previous steps. In the fourth step, the residual fraction was extracted (Rauret et al., 1999). The residue from the third step (Fig. 27) was digested using 9 ml nitric acid – HNO_3 (65%) and 1 ml hydrogen peroxide – H_2O_2 (30%). After cooling, the digests were filtered, adjusted to 50 ml with deionized Milli-Q water, and stored in polypropylene bottles until analysis. A procedure blank (reagents without any sample) was subjected to the same digestion procedure. The quality of the analytical data for the sequential extraction procedure was assessed by carrying out analyses of the certified reference materials BCR-701 and CRM483. BCR-701 is a lake sediment certified for extractable metal contents in the three steps of the modified BCR sequential extraction procedure and indicative values for aqua regia extraction (Pueyo et al., 2001). CRM483 is a sewage sludge amended soil that has indicative values for Cd, Cr, Cu, Ni, Pb and Zn in the 3 steps of the BCR extraction procedure. Additionally, the BCR sequential extraction procedure was also applied to the reference soil SRM 2710 (Gillis, 1997), which is certified for its total element concentrations.

Fig. 24 Centrifugation of the extracts

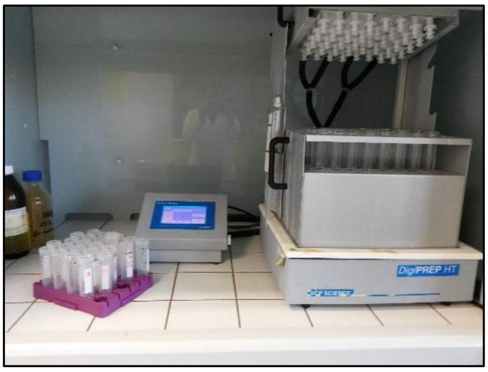


Fig. 25 Digestion of the mixture at 85 ± 2°C

Fig. 26 Reduction of volume of the residue between 2 – 3 mL

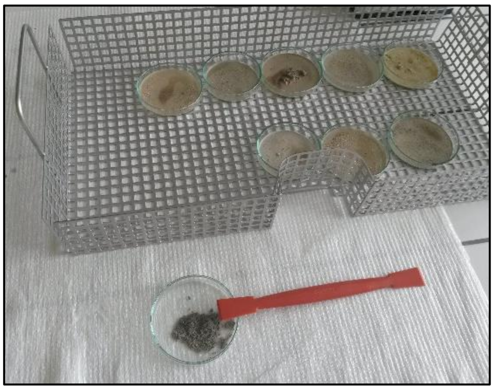


Fig. 27 The residue from the third step

Determination of metal by AAS

The concentrations of metals in the extracts were determined using flame 55 Atomic Absorption Spectrometer (F55AAS) (Fig. 28) and graphite tube atomizer AA (GTA 120) (Agilent technologies) (Fig. 29). The relative standard deviations for triplicate measurements were lower than 10 % for the FAAS and lower than 15 % for the GFAAS. Replicate analyses of these reference materials showed good accuracy, with recovery rates for metals between 90 and 108 %. As an additional check on the analytical procedure, selected samples were digested and optimize. The accuracy for FAAS is 100 – 95% (measurement is every 10 second) and the accuracy for GFAAS is 100 – 90% (it must be replicate).

Fig. 28 Flame 55 Atomic Absorption Spectrometer (F55AAS) and samples

Fig. 29 Graphite Tube Atomizer AA (GTA 120)

3.4. Toxic metals hazard identification

The results from chemical analyses are used as an indicator of the potential for a toxic metal hazard in the aquatic ecosystems.

Concentrations of toxic metals in water were compared with the Environmental Quality Standard (EQS) of Government Order 229/2007 (Nábělková & Komínková, 2012). An estimation of bioavailability and the hazard of metals in sediments was based on several indexes: Distribution Coefficient (K_d) is the ratio between a pollutant concentration in sediment (c_s) and its concentration in water (c_w):

$$K_d = \frac{C_s}{C_w} \ [\mathrm{L.kg^{-1}}]$$

K_d gives information about what medium (water or solid phase) is crucial for the risk assessment (U.S. EPA, 1999; Nábělková & Komínková, 2012). This distribution coefficient is one of the most important parameters for assessing the migration potential of a contaminant present in the liquid phase that is in contact with sediment or suspended matter. Values of $\log K_d > 5$ are calculated for elements/compounds that prefer binding to solid phases and only marginally migrate into liquid phase (Nábělková & Komínková, 2012). On the contrary, values of $\log K_d < 4$ characterize chemicals more easily released from solid phases and $\log K_d < 3$ chemicals present preferentially in the liquid phase (Komínková, 2001). Hazard Quotient (HQ) characterizes the danger of a pollutant for the aquatic environment by comparison with a particular EQS. To evaluate the pollutant concentration in sediment, the following equation can be used:

$$HQ = \frac{C_s}{EQS}$$

where c_s is the measured concentration of a pollutant in sediment and EQS is a suitable criterion for the assessment of sediment quality. In this study, the US Environmental Protection Agency (USEPA) benchmark Threshold Effect Concentration (TEC) and Probable Effect Concentration (PEC) were used because of the absence of suitable criteria in Czech national legislation. An ecological hazard is indicated for $HQ > 1$ (Barnthouse et al., 1982 ex. Nábělková & Komínková, 2012). Based on the value of HQ, it is possible to predict changes in benthic community composition by categorization according to Clements et al., (2000): $HQ < 1$ indicates unpolluted locality with no or reversible effect on aquatic organisms; $1 < HQ < 2$ shows low pollutant load with no acute danger for organisms; $2 < HQ < 10$ signalizes intermediate pollutant load with fatal effect to sensitive species and $HQ > 10$ means high pollutant load reflecting on benthic diversity decrease (Nábělková & Komínková, 2012).

Mobility Factor (MF). The bioavailability and consequently the hazard of problematic metals (Cd, Cu, Pb and Zn) according to their binding strength in sediment are evaluated by MF, as described by Kabala and Singh (2001):

$$MF = \frac{C_{1+2}}{C_s} . 100 [\%]$$

where c_{1+2} is the concentration of a pollutant bound to the two most available geochemical sediment fractions (exchangeable and carbonates) and c_s is the overall concentration of a pollutant in sediment (the sum of concentrations in all geochemical fractions: exchangeable, carbonates, Fe/Mn oxides, organic matter and residual). This factor expresses the ability of the sediment to release mobile (the most toxic) forms of pollutants during changes of conditions in the aquatic environment (Nábělková & Komínková, 2012).. Risk Assessment Code (RAC). Based on values of MF, the risk of sediment contaminated by Cd, Cu, Pb and Zn for water environment was quantified by an RAC (Table 3) (Jain, 2004; Hnatukova, 2007).

Biota sediment accumulation factor (BSAF). The bioavailability of toxic metals was verified directly by analyzing their concentrations in aquatic organisms (benthos). The ability of benthic organisms to accumulate metals from sediments is indicated by the BSAF, calculated as:

$$BSAF = \frac{C_B}{C_S}$$

where c_B is the concentration of a metal per dry weight of an organism and c_s is the concentration of that metal in sediment (Rand, 1995 ex. Nábělková & Komínková, 2012). Furtehrmore, the Directive 2008/105/EC of Environmental Quality Standards (EQS) entail values for various chemicals in biota (Gheorghe et al., 2017).

Table 3 Quantifying the Risk of Contaminated Sediment for the Water Environment by RAC

RAC	**1**	**2**	**3**	**4**	**5**
MF	<1%	1-10%	11-30%	31-50%	>50%
Risk	no	low	intermediate	high	very high

Source: Nábělková & Komínková, 2012.

3.5. Statistical analyses

The concentrations of toxic metals in sediments obtained from digested procedure – Autumn season, were tested in relation to the type of springs (rheocrene I, limnocrene (L), helocrene (H) and antrophogenic (A)); type of geology: (car 1 (Carboniferous (Early Permian), biotite-monzogranites), car 2 (Carboniferous (Early Permian), muscovite-biotite monzogranites), cra 1 (Cretaceous, quartz sandstones), cra 2 (Cretaceous, sandstones), cam 1 (Cambrian, augen gneisses), cam 2 (Cambrian, limestones), cam 3 (Cambrian, sericite quartzites) and dev 1 (Devonian, limestones)) and levels of sediment fractions (<0.061 mm, 0.206-0.061 mm, 0.609-0.206 mm and > 0.609 mm total fraction). The concentrations of toxic metals in sediments, obtained from sequential extraction procedure, were tested in relation to the type of springs (R, L, H and A); type of geology: (car 1, car 2, cra 1, cra 2, cam 1, cam 2, cam 3 and dev 1) and chemical fractionation (Mobile/exchangeable, reducible, oxidizable and residuals).

The concentration of toxic metals (Cu, Cd, Zn and Pb) was used as a response variable in sediments, while type of springs, type of geology and fraction levels (sediment fractions and geochemical fractions) were used as predictors for sediment. The depence of the response variable on predictors was tested using generalized linear models (GLM) in which the response variable wa approximated by gamma distribution with a logarithmic link function (Rinaldi et al., 2017 ex. Crawley, 2013). The analyses of covariance between the concentration of toxic metal in biota and sediments was tested using linear models (LM). The statistical analyses were conducted using Rstudio.

IV. Results:

4.1. Physical chemical water parameters

Conductivity of the springs water implies the presence of soluble forms, and more conductivity is the sign of high presence of ions. Conductivity related to the soluble forms of solids directly. The mean conductivity (EC) in studied sites was varying from 78.7 µS/cm at DN1012 site to 767 µS/cm in spring season, and from 93 µS/cm at DN1012 site to 907 µS/cm in autumn season. The PH of the samples in different springs was 5.49 at EN1020 site to 8.02 at EN1011 site in spring season, and was 4.62 at EN1004 site to 7.31 at EN1011 site in autumn season. We observed a pH decrease in autumn season, while in spring period, 11 springs of 20 were neutral and basic; during autumn period, only 3 springs were basic, it means in autumn season acid pH predominated. Temperature (T) of the water samples in different sites in spring season was 7.3 at EN1022 site to 14.1 at EN1021 site and in autumn season was 6.6 at EN1006 site to 11.6 at EN1002 site.

The concentration of DO in studied sites was 4.11 mg. L^{-1} at BS1018 site to 10.83 mg. L^{-1} at EN1022 site in spring season, and was 3.19 mg. L^{-1} at DN1012 site to 11.28 mg. L^{-1} at DN1010 site in autumn season. The results reflected a decreasing DO concentration in spring sites from spring to autumn season, most of them did not vary significately; although, DN1012 decreased (7.92 - 3.19 mg. L^{-1}) in more than 50% from spring to autumn season (Table 4).

Table 4. Physical and chemical water parameters of sampling sites

Springs			1. collection - 2016					2.collection - 2016				
Site number	**Site name**	**Localities**	**Date**	**pH**	**Conductivity µS/cm**	**Temerature °C**	**LOD mg/l**	**Date**	**pH**	**Conductivity µS/cm**	**Temerature °C**	**LOD mg/l**
1	BS1007	Petrovice - Kněžický potok	29/06/2016	Unsampled				25/10/2016	Unsampled			
2	BS1016	Radvanec	29/06/2016	6.36	111.9	9.40	8.92	25/10/2016	5.41	123.6	10.10	8.70
3	BS1017	Ortel	29/06/2016	7.36	201.4	12.00	8.11	25/10/2016	6.16	192.1	9.90	8.88
4	BS1018	Cesta ke Krompachu	21/06/2016	7.05	152.6	12.40	9.52	26/10/2016	Unsampled			
5	BS1019	Krompach - dřevěný jímací obj.	21/06/2016	7.49	192.4	9.30	4.11	26/10/2016	6.50	227.0	9.30	3.75
6	BS1027	Heřmanický vodopád	21/06/2016	7.73	117.3	7.40	9.20	26/10/2016	6.62	128.0	7.80	9.25
7	DN1010	Kryštofovo údolí - vodárna	18/07/2020	6.92	131.4	9.00	8.42	31/10/2016	6.60	251.0	8.80	11.28
8	DN1012	Vítkovský potok	11/07/2016	6.76	78.7	9.20	7.92	31/10/2016	5.68	93.0	10.80	3.19
9	DN1013	Kryštofovo údolí	18/07/2016	Unsampled				31/10/2016	Unsampled			
10	EN1001	Na Skřivanech	11/07/2016	7.22	158.5	11.50	9.54	24/10/2016	6.24	137.7	10.00	10.24
11	EN1002	Jizerská kompost	11/07/2016	6.56	408.0	13.80	5.41	24/10/2016	5.52	434.0	11.60	4.49
12	EN1004	Skautská studánka - Liberec	11/07/2016	6.13	85.2	9.30	9.45	24/10/2016	4.62	103.3	8.80	8.83
13	EN1005	Terasy Ještědu - Frantina studánka	24/06/2016	7.95	276.0	7.80	7.69	24/10/2016	7.17	313.0	6.70	6.69
14	EN1006	Pramen lesních panen	24/06/2016	7.84	183.5	11.70	7.69	24/10/2016	7.17	221.0	6.60	10.68
15	EN1011	Jiříčkov	24/06/2016	8.02	334.0	12.50	10.45	24/10/2016	7.31	385.0	9.70	10.27
16	EN1015	Harcov - Liberec	11/07/2016	6.63	767.0	11.20	9.44	24/10/2016	5.56	907.0	10.60	8.74
17	EN1020	Milíře	11/07/2016	5.49	116.5	10.40	7.22	31/10/2016	Unsampled			
18	EN1021	Mokřad Ještěd	24/06/2016	6.27	439.0	14.10	7.66	24/10/2016	6.25	566.0	8.40	9.04
19	EN1022	Ostašovský potok - Ještěd	24/06/2016	6.95	258.0	7.30	10.83	24/10/2016	6.34	261.0	8.00	10.20
20	ES1008	Vápno	24/06/2016	7.45	762.0	11.50	9.57	24/10/2016	6.64	684.0	10.10	9.16
21	ES1009	Lesnovek	24/06/2016	7.58	520.0	9.80	9.72	24/10/2016	6.80	576.0	9.30	9.40
22	ES1014	Janův důl	18/07/2016	7.47	486.0	12.60	8.25	24/10/2016	6.07	629.0	8.70	6.62

4.2. Accumulation of toxic metals in sediments (Determination by AAS):

Toxic metals concentration in the pseudototal fractions (Microwave digestion procedure)

Although more elements were analysed, the discussion will mainly focus on Zn, Pb, Cd and Cu. The concentration of Zn, Pb, Cd and Cu among the different pseudototal fractions (first < 0.061 mm, second 0.206-0.061 mm, third 0.609-0.206 mm, and fourth > 0.609 mm) in sediment samples from the microwave digestion procedure were obtained from the two periods spring and autumn season, but only the 50% of samples was possible to obtain all pseudototal fractions for both seasons, because the material content of the rest of samples was very low, due to the sediment of springs were mixed with stones or they associated high organic material content; however, all pseudototal concentrations (>0.609mm fraction) of sediment samples from both season (spring and autumn) were analized.

Cu and Pb are the most abundant toxic metals in the springs sediments. The most occurrence of Cu among the 20 samples was in the first sediment fraction 65%, during spring season 70%. The 4 samples with the highest concentrations of Cu were EN1021 (629.26 mg.kg^{-1}), EN1022 (606.5 mg.kg^{-1}), EN1020 (91.7 mg.kg^{-1}) and ES1009 (77.28 mg.kg^{-1}), and the lowest Cu concentrations were BS1027 and BS1019, both present concentratios under the detection limit (DL), DL in the last three sediment fraction in autumn sesason. The most occurrence of Pb among the 20 samples was in the first and third sediment fraction, 30% respectively for both, during spring season 80%. The 4 samples with elevated concentrations of Pb were EN1006 (87.02 mg.kg^{-1}), EN1021 (83.67 mg.kg^{-1}), ES1009 (81.99 mg.kg^{-1}) and BS1016 (71.27 mg.kg^{-1}), whereas the Pb content of sample ES1008 was under DL.

The most occurrence of Zn among the 20 samples was in the first sediment fraction (40%), during autumn season (55%). The 4 samples with elevated concentrations of Zn were BS1019 (158.31 mg.kg^{-1}), EN1022 (157.1 mg.kg^{-1}), ES1009 (149.79 mg.kg^{-1}) and BS1018 (143.48 mg.kg^{-1}), and the lower concentration were ES1008 (5.54 mg.kg^{-1}) and BS1016 (6.22 mg.kg^{-1}), both from third sediment fraction in autumn sesason). Very high Cd concentrations were detected in samples EN1022 (3.59 mg.kg^{-1}), EN1006 (1.59 mg.kg^{-1}), ES1014 (1 mg.kg^{-1}) and EN1021

(0.97 mg.kg^{-1}), whereas the Cd-content of sample ES1008 was significantly lower (0.006 mg.kg^{-1}).

EN1004 site displayed the highest acid pH, 6.13 and 4.62 in spring and autumn season respectively, among the springs sites, while EN1011 displayed the highest basic pH, 8.02 and 7.31 in spring and autumn season respectively. The pH in ES1014 site was basic 7.47 in spring season and acid 6.07 in autumn season. Toxic metals concentration in pseudototal fractions of EN1004 (Fig. 30) registered the lowest concentrations in 0.609-0.206mm fraction for Cu (2.93 mg.kg^{-1}), Pb (6.05 mg.kg^{-1}), Zn (13.08 mg.kg^{-1}) and Cd (0.05 mg.kg^{-1}) in spring season, and the highest concentration in <0.061mm fraction for Pb (66.6 mg.kg^{-1}), Zn (88.5 mg.kg^{-1}) and Cd (0.30 mg.kg^{-1}) in autumn season and the highest Cu concentration (10.78 mg.kg^{-1}), in <0.061mm fraction in spring season.

Toxic metals concentration in pseudototal fractions of ES1014 (Fig. 31) registered the lowest concentrations in 0.609-0.206mm fraction for, Zn (37.68 mg.kg^{-1}) and Cd (0.47 mg.kg^{-1}) in autumn season, the lowest Cu concentration (2.93 mg.kg^{-1}) was found in 0.206-0.061mm fraction in spring season, the lowest Pb concentration (17.71 mg.kg^{-1}) was found in 0.609-0.206mm fraction in autumn season. The highest Cu concentration (10.93 mg.kg^{-1}) was found in 0.609-0.206mm fraction in spring season. The highest Pb concentration (45.96 mg.kg^{-1}) was found in >0.609mm fraction in autumn season. The highest concentrations of Zn (90.86 mg.kg^{-1}) and Cd (1.00 mg.kg^{-1}) were found in 0.206-0.061mm fraction in spring season.

Total Cu concentrations in pseudototal fractions (Fig. 32) in spring season ranged from BS1019 (0.70 mg.kg^{-1}) to EN1020 (91.70 mg.kg^{-1}); in autumn season ranged from BS1019 and BS1027 (close to 0 mg.kg^{-1}) to EN1020 (54.77 mg.kg^{-1}). EN1021 (44.96 mg.kg^{-1}), and EN1020 (91.70 mg.kg^{-1}) sites during spring season and EN1021 (41.33 mg.kg^{-1}), EN1022 (43.05 mg.kg^{-1}), and EN1020 (54.77 mg.kg^{-1}) sites during autumn season, exceeded the EQS given by USEPA (31.6 mg Cu kg^{-1}).

Total Pb concentrations in pseudototal fractions (Fig. 33)in spring season ranged from ES1008 (close to 0 mg.kg^{-1}) to EN1011 (47.31 mg.kg^{-1}); in autumn season ranged from BS1018 (2.81 mg.kg^{-1}) to EN1021 (83.67 mg.kg^{-1}). EN1022 (41.58 mg.kg^{-1}), and EN1011 (47.31 mg.kg^{-1}) sites during spring season and ES1009 (38.88 mg.kg^{-1}), DN1010 (42.96 mg.kg^{-1}), EN1015 (44.65 mg.kg^{-1}), ES1014 (45.96 mg.kg^{-1}), DN1012 (46.72 mg.kg^{-1}), EN1011 (47.47 mg.kg^{-1}), EN1004 (58.68 mg.kg^{-1}), EN1022 (62.37 mg.kg^{-1}), and EN1011 (83.67 mg.kg^{-1})sites during autumn season, exceeded the EQS given by USEPA (35.8 mg Pb kg^{-1}).

Total Zn concentrations in pseudototal fractions (Fig. 34) in spring season ranged from ES1008 (9.15 mg.kg^{-1}) to EN1022 (115.93 mg.kg^{-1}); in autumn season ranged from ES1008 (11.32 mg.kg^{-1}) to EN1022 (134.71 mg.kg^{-1}). EN1015 (128.95 mg.kg^{-1}), and EN1022 (134.71 mg.kg^{-1}) sites during autumn season exceeded the EQS given by USEPA (121 mg Zn kg^{-1}).

Total Cd concentrations in pseudototal fractions (Fig. 35) in spring season ranged from ES1008 and ES1009 (0.01 mg.kg^{-1}) to EN1022 (3.56 mg.kg^{-1}); in autumn season ranged from DN1010 (0.02 mg.kg^{-1}) to EN1022 (3.51 mg.kg^{-1}). EN1006 (1.59 mg.kg^{-1}), and EN1022 (3.56 mg.kg^{-1}) sites during spring season and EN1022 (3.51 mg.kg^{-1}) site during autumn season exceeded the EQS given by USEPA (0.99 mg Cd kg^{-1}).

In average the highest pseudototal concentrations for toxic metals, Cu (430.48 mg.kg^{-1}), Zn (134.71 mg.kg^{-1}), Pb (62.37 mg.kg^{-1}) and Cd (3.51 mg.kg^{-1}) were found in EN1022 during autumn season; and the lowest pseudototal concentrations for toxic metals, Cu (1.21 mg.kg^{-1}), Zn (9.15 mg.kg^{-1}), Pb (close to 0 mg.kg^{-1}) and Cd (0.01 mg.kg^{-1}) were found in ES1008 during spring season.

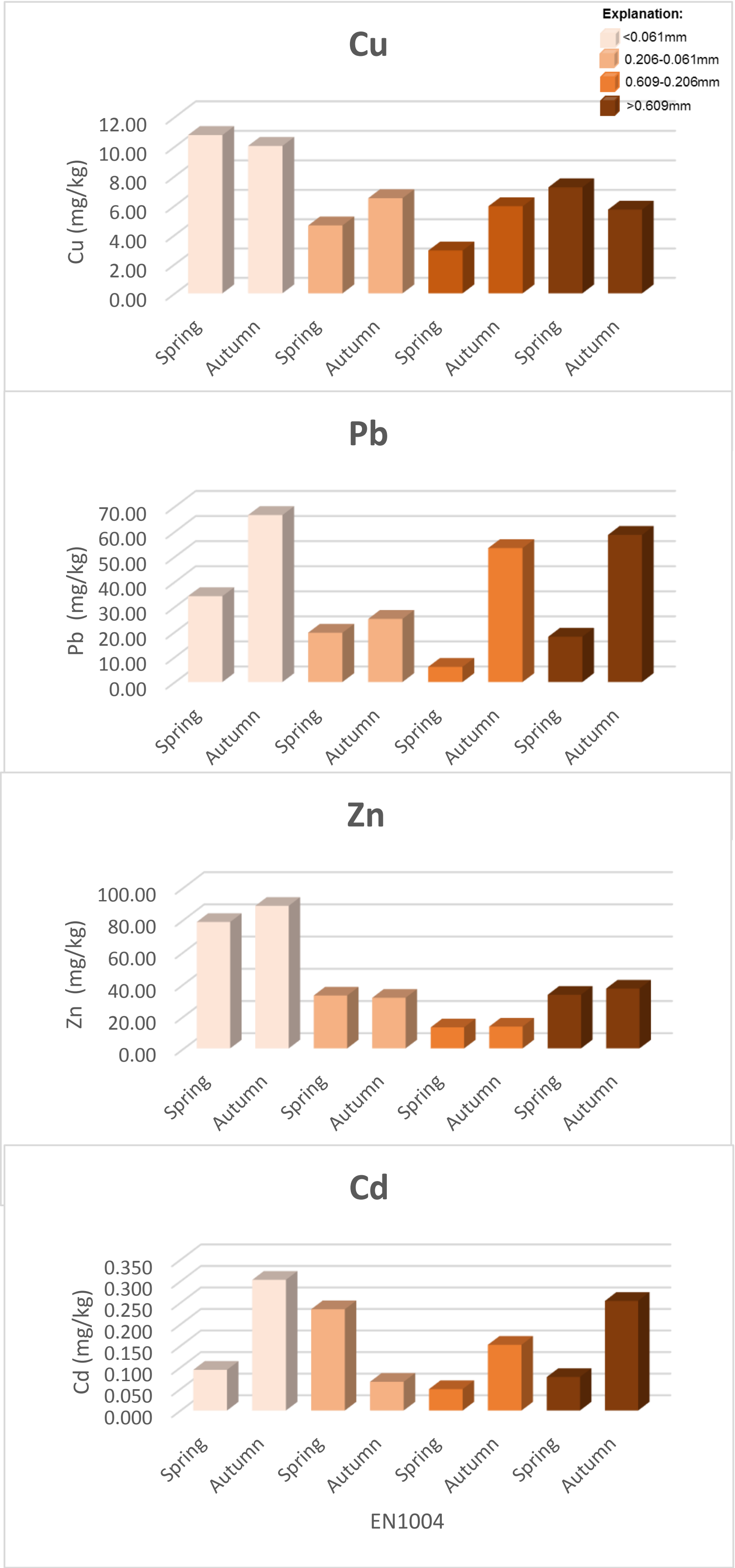


Fig. 30 Metals concentration (mg.kg^{-1}) among the sediment fractions in EN1004 site.

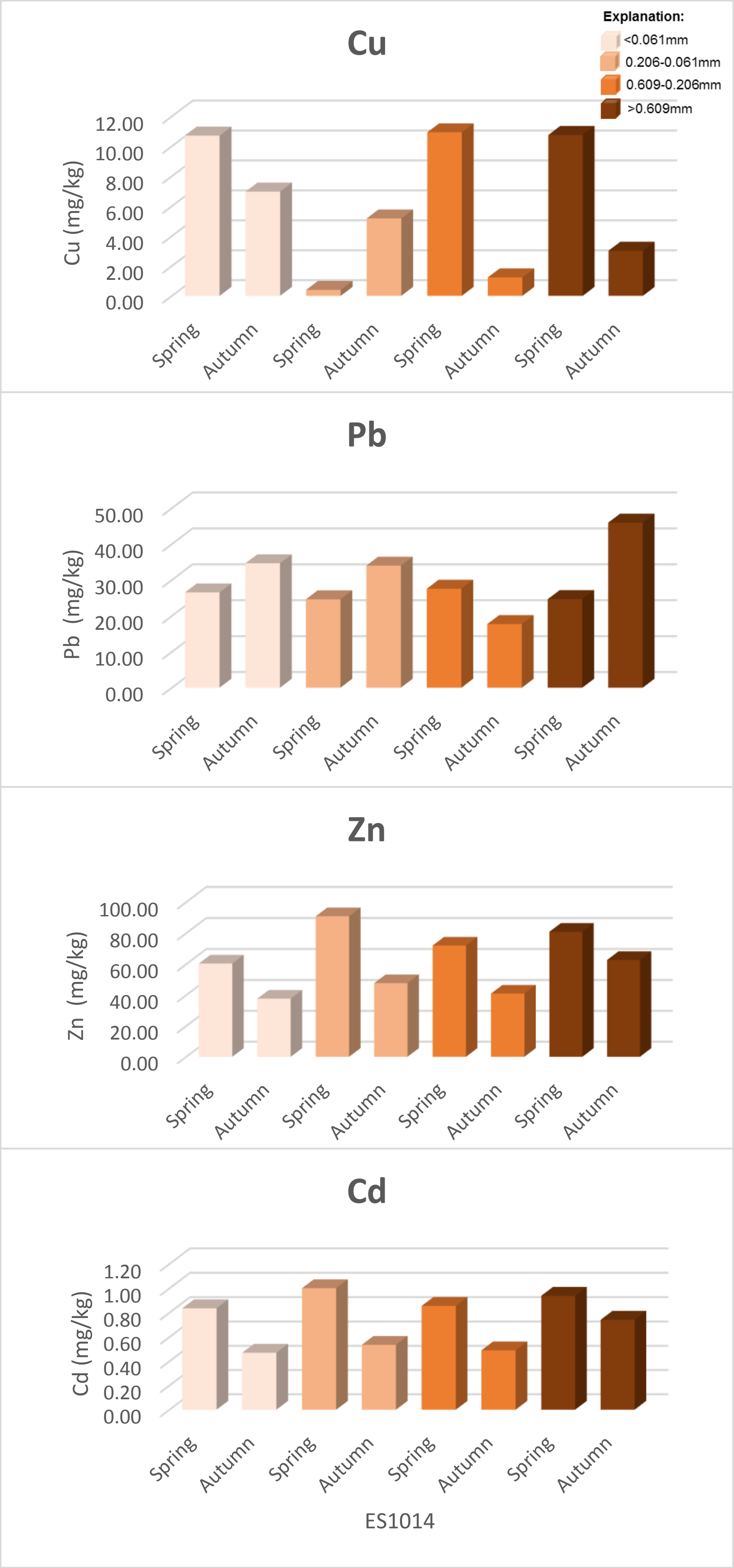


Fig. 31 Metals concentration (mg.kg^{-1}) among the sediment fractions in ES1014 site.

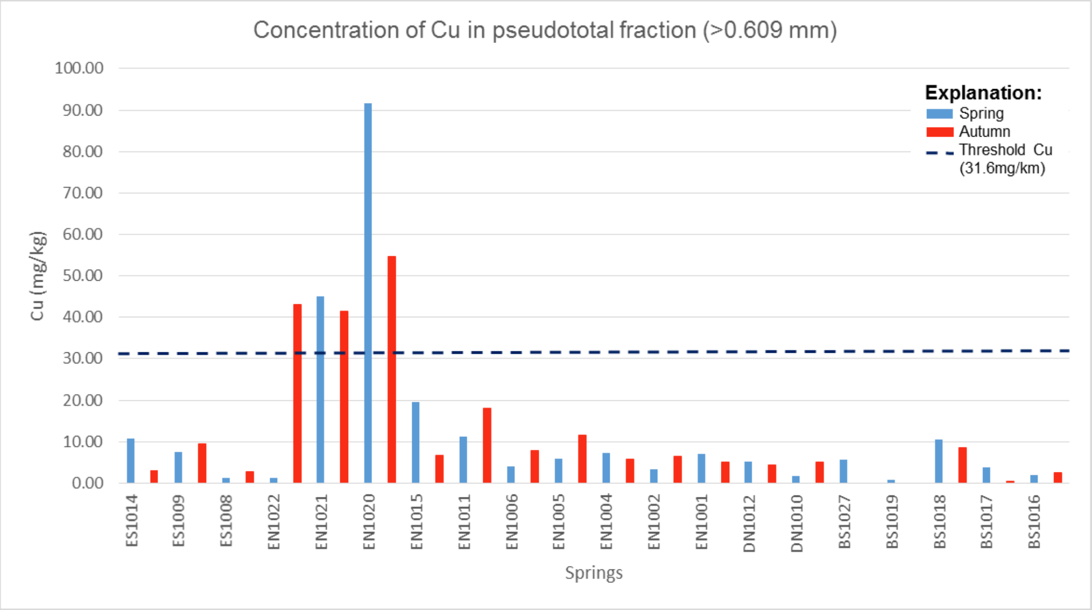


Fig. 32 Concentration of Cu in pseudototal fraction of sampling sites.

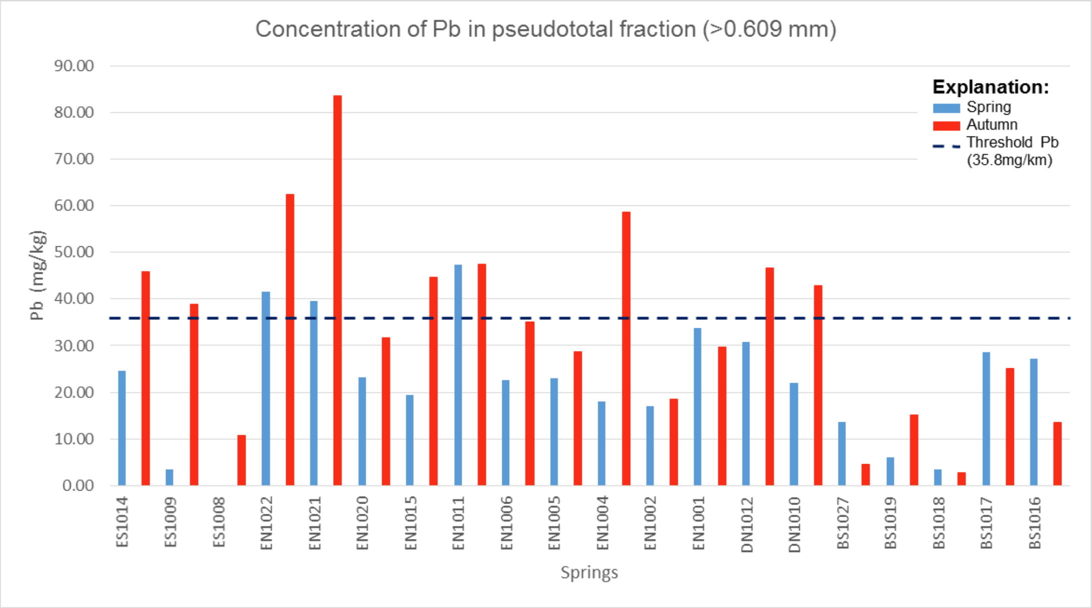


Fig. 33 Concentration of Pb in pseudototal fraction of sampling sites.

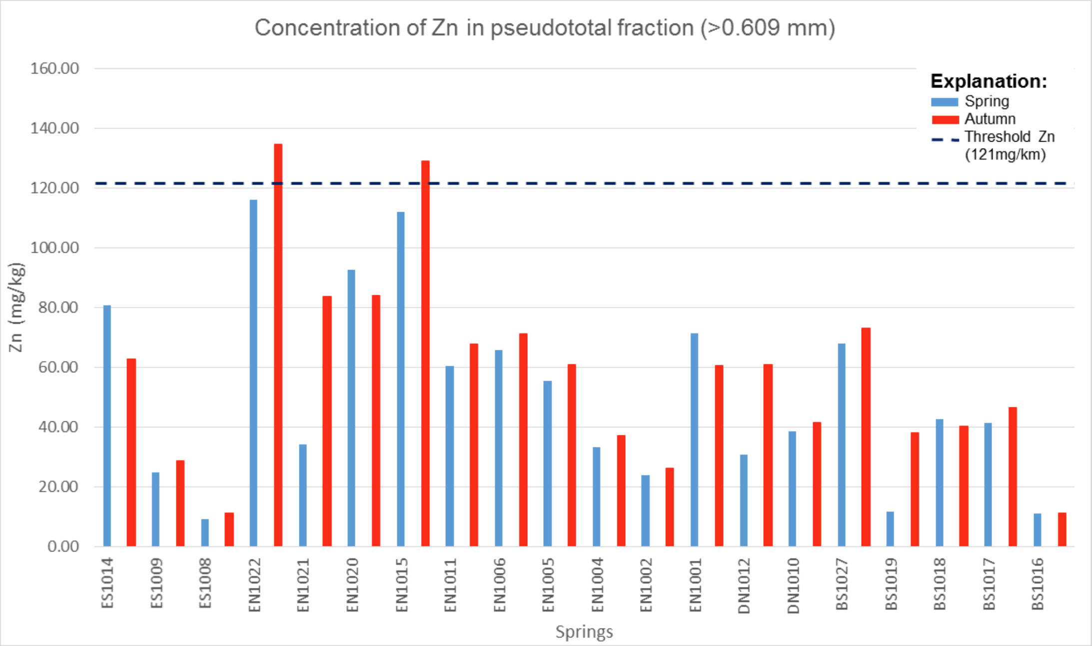


Fig. 34 Concentration of Zn in pseudototal fraction of sampling sites.

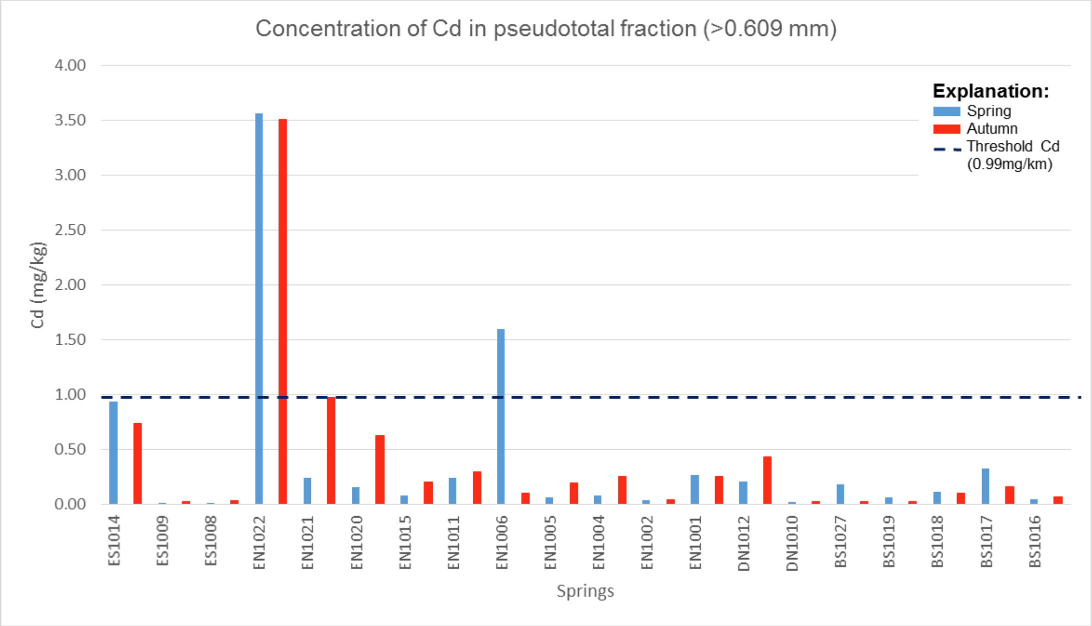


Fig. 35 Concentration of Cd in pseudototal fraction of sampling sites.

Toxic metals concentration and distribution in the geochemical Fractions (BCR Sequential extraction)

We used an optimized BCR sequential extraction procedure to determine the operationally defined fraction. The concentrations (mg.kg^{-1}) of Cd, Cu, Pb and Zn in the different geochemical fractions and their proportions (%) of mobile/exchangeable, reducible, oxidizable, and residual fractions from the BCR sequential extraction procedure wre analized.

The results showed the Cd concentration (Fig. 36) in most of sediments was strongly retained in the exchangeable fraction, in the chart this fraction ranged from 11% at BS1027 site (0.01 mg Cd kg^{-1}) to 88% at EN1004 site (0.07 mg Cd kg^{-1}). EN1022 site (real concentration in the exchangeable fraction: 2.73 mg Cd kg^{-1}, in the chart is divided by 10, Fig. 36), represented the highest Cd concentration (5.49 mg.kg^{-1}), 50% of Cd concentration in this site is retaind in the exchangeable fraction, and 42% is retaind in the reducible fraction. Cu concetration (Fig. 37) in all sediments are mostly present in the oxidizable and reducible fraction, with the exception at BS1019 site (6.53 mg Cu kg^{-1}), which is mostly retained (55%) in the residual fraction; copper was associated with organic matter, and its mobility was controlled by the concentration and degradation of the organic fraction. The oxidizable fraction ranged from 13% at EN1004 site (3.41 mg Cu kg^{-1}) to 80% at ES1014 site (6.96 mg Cu kg^{-1}); the reducible fraction ranged from 3% at BS1019 site to 62% at EN1004 site. Higher levels of the reducible fraction of Cu can be attributed to the affinity of this metal to metals associated with manganese and iron oxides or hydroxides (Wali et al., 2014). Although the Cu concentration of the sediments mentioned above are very important, they have low Cu concentration and does not overpass the threshold of toxic effect concentrations (31.6 mg Cu kg^{-1}). Sediments from EN1022 site (355.89 mg Cu kg^{-1}, in the chart is divided by 6, Fig. 34) hugely overpass this threshold, 52% of Cu concentration in this site is hold in the reducible fraction, 20% in the exchangeable fraction, and it represents the highest Cu concentration in this fraction among the sediments, while EN1020 (44.25 mg Cu kg^{-1}) and EN1021 (51.25 mg Cu kg^{-1}) sites also contain high concentrations of Cu, they are heavily bound to the oxidizable fraction (EN1020 – 64% and EN1021 – 77%) and the residual fraction (EN1020 – 28% and EN1021 – 18%), and less than 10% is retained in the first two fractions, thus, they are bound to organic matter or in the form of metal sulfides (oxidizable fraction), and incorporated into crystalline mineral lattices, and refractory unoxidized organic matter as undissolved amorphous iron oxide (residual fraction).

Highest percentages of Pb concentration were found in residual fraction(Fig. 35), this fraction ranged from 6.8% at site BS1017 (3.09 mg Pb kg^{-1}) to 92% at site BS1027 (9.24 mg Pb kg^{-1}). This metal is strongly bound in the crystal lattices of minerals and, consequently, have low mobility. Because of that, they will not be released into the environment. Although, slightly Pb concentrations were found in the exchangeable fraction; 24% at ES1009 site (1.74 mg Pb kg^{-1}), 21% at EN1005 site (4.12 mg Pb kg^{-1}) and 20% at BS1017 site (3.09 mg Pb kg^{-1}); they represent the lowest Pb concentration among the sediments. There is a similar distribution between the three sediments with the highest Pb concentrations, their concentrations were strongly retained in the reducible fraction; 63% at EN1002 site (33.07 mg Pb kg^{-1}), 48% at EN1015 site (34.58 mg Pb kg^{-1}) and 48% at DN1012 site (25.1 mg Pb kg^{-1}); but in the exchangeable fraction, their concentrations were very low.

In most sediment samples the exchangeable fraction of Zn raged from 9% at EN1011 site (67.87 mg Zn kg^{-1}) to 27% ES1009 site (43.54 mg Zn kg^{-1}), except for sediments from location EN1004 (29.41 mg Zn kg^{-1}) and EN1005 (58.88 mg Zn kg^{-1}), where 45% and 36% of total Zn was determined in the exchangeable fraction, respectively. The residual fraction of Zn ranged from 7% at EN1004 site (29.41 mg Zn kg^{-1}) to 40% at two sites: EN1006 (86.8 mg Zn kg^{-1}), EN1022 (117.9 mg Zn kg^{-1}).The oxidizable fraction of Zn ranged from 20% at BS1017 site (39.79 mg Zn kg^{-1}) to 48% at BS1019 site (32.45 mg Zn kg^{-1}), and the reducible fraction of Zn ranged from % at BS1019 site (32.45 mg Zn kg^{-1}) to 42% at BS1027 site (22.08 mg Zn kg^{-1}). Although, in most sediment samples the Zn concentration (Fig. 36) is relatively equal distributed into the different geochemical fractions, the reducible fraction of Zn, which is bound to organic matter, predominated.

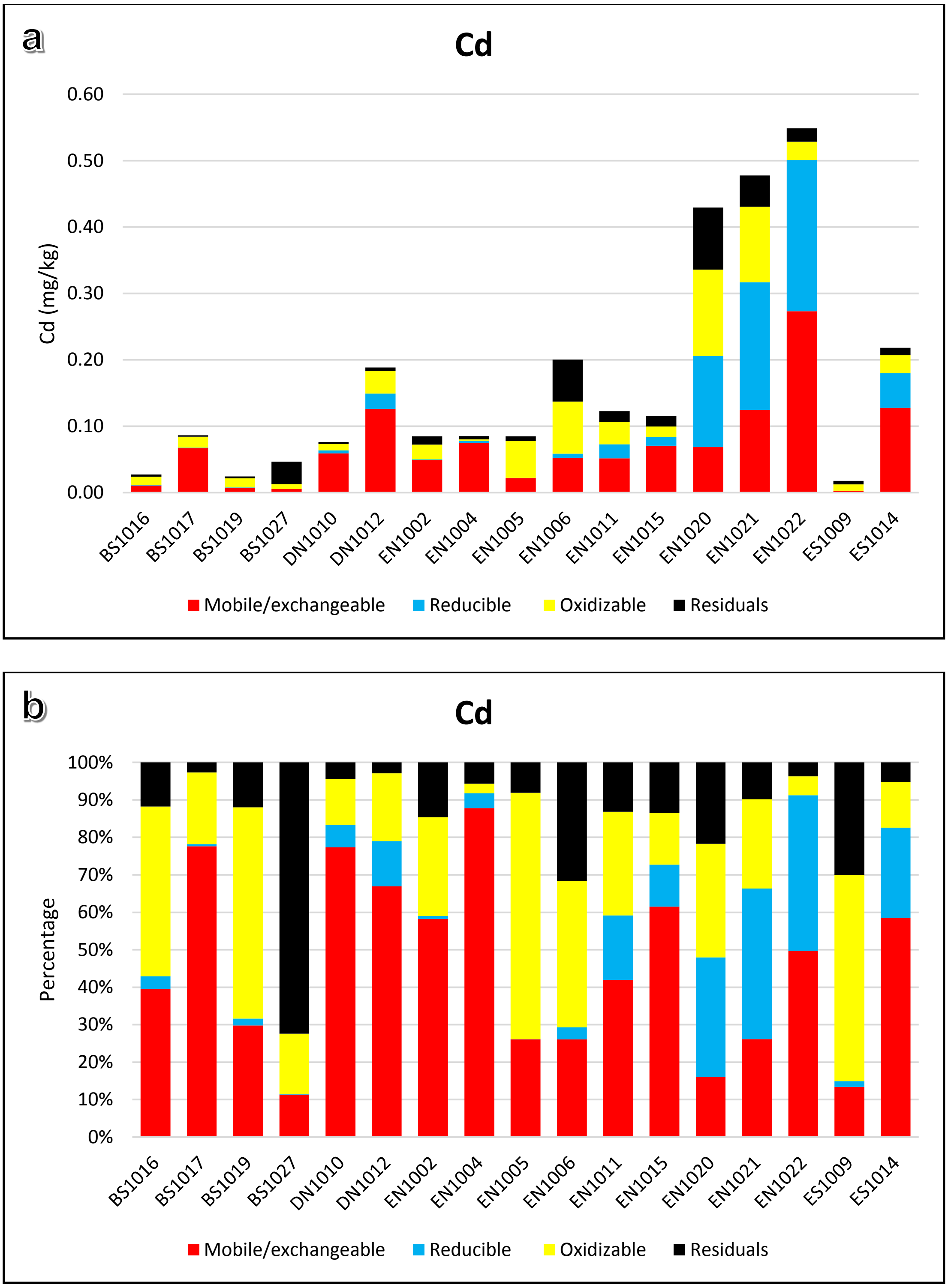


Fig. 36 (**a** Chemical fractionation of Cd (mg.km^{-1}), **b** Cd distribution (%) in the geochemical fractions)
Note Cd concentration in EN1022 was divided by 10

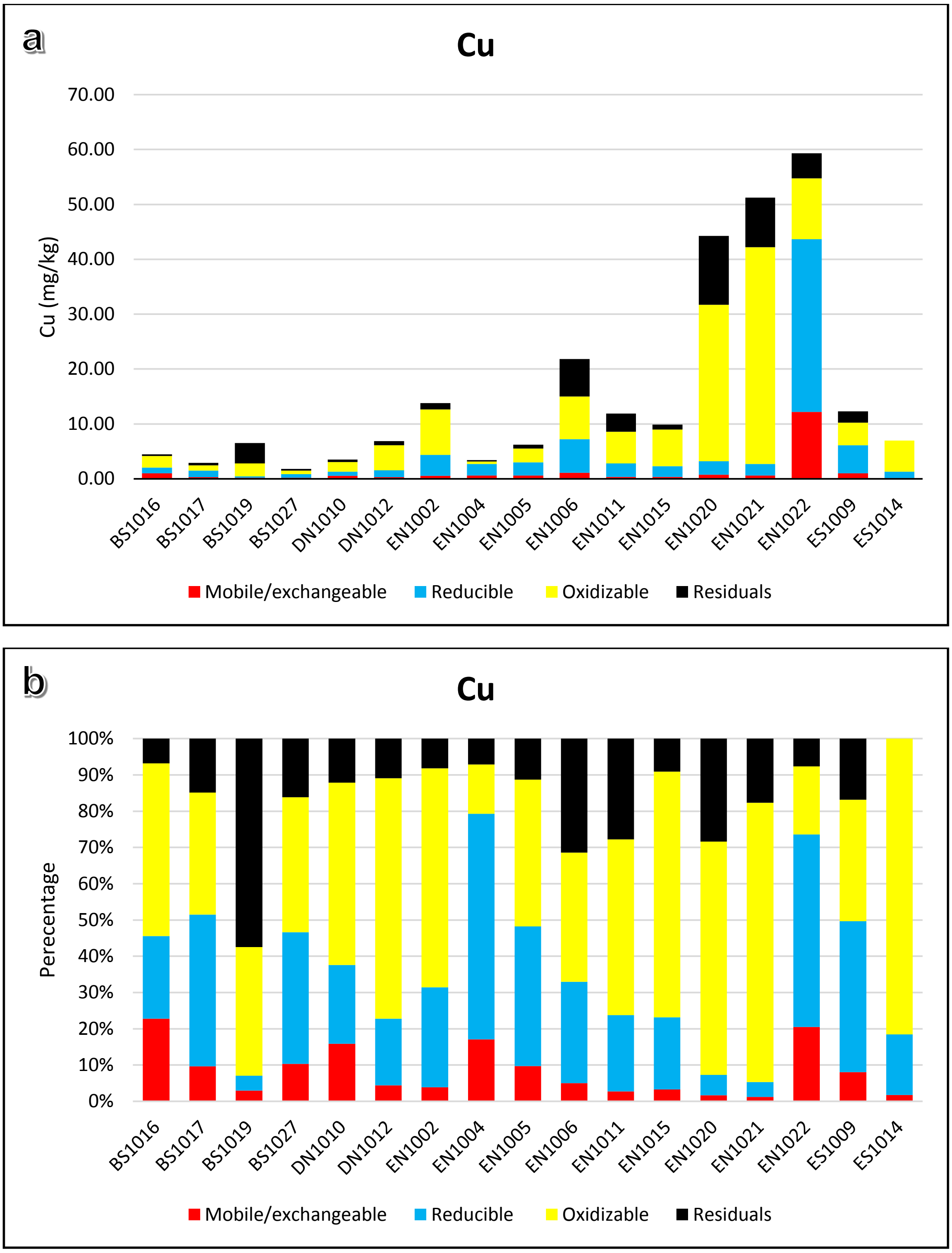


Fig. 37 (**a** Chemical fractionation of Cu (mg.km^{-1}), **b** Cu distribution (%) in the geochemical fractions)
Note: Cu concentration in EN1022 was divided by 6.

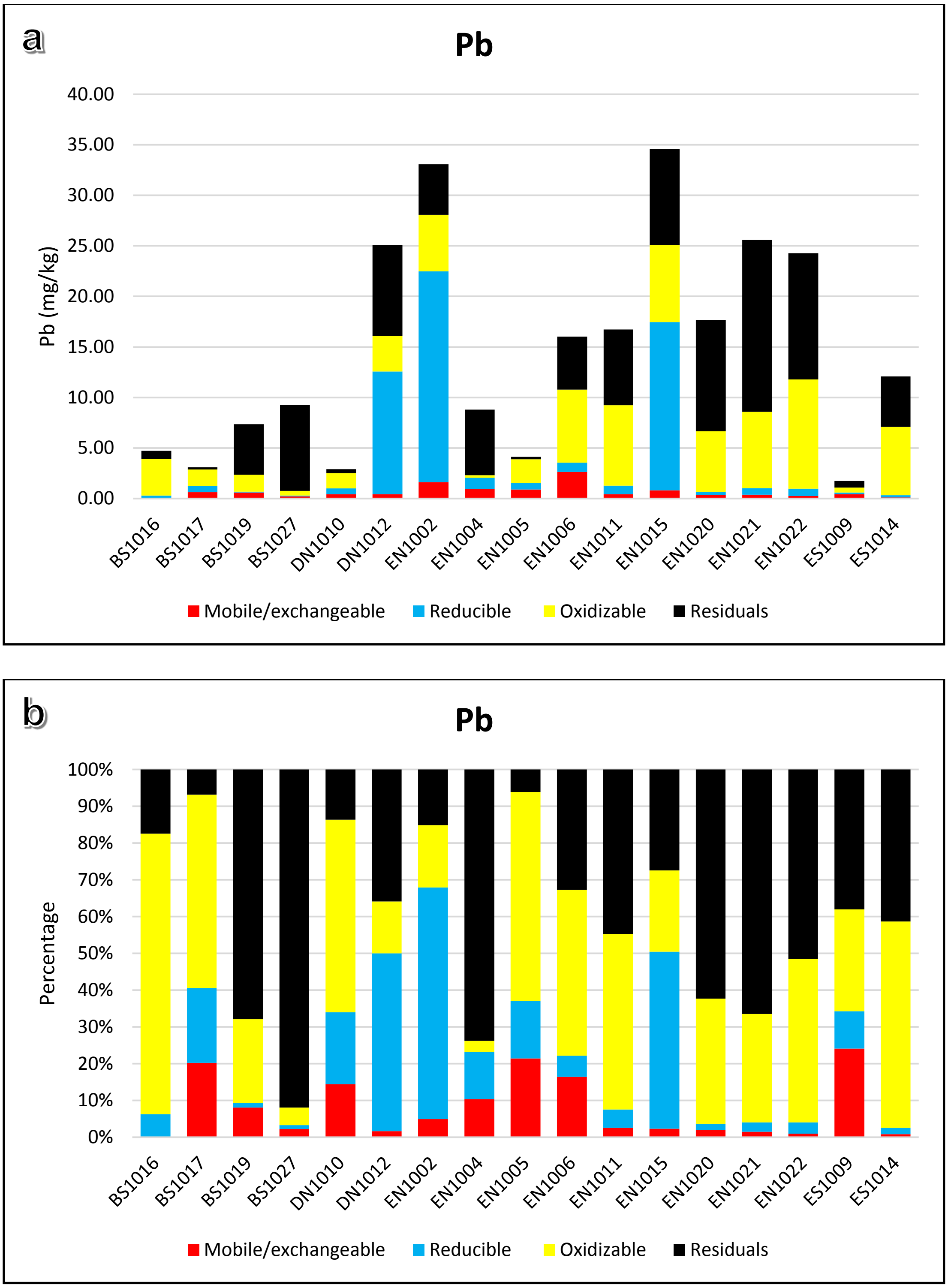


Fig. 38 (**a** Chemical fractionation of Pb (mg.km^{-1}), **b** Pb distribution (%) in the geochemical fractions)

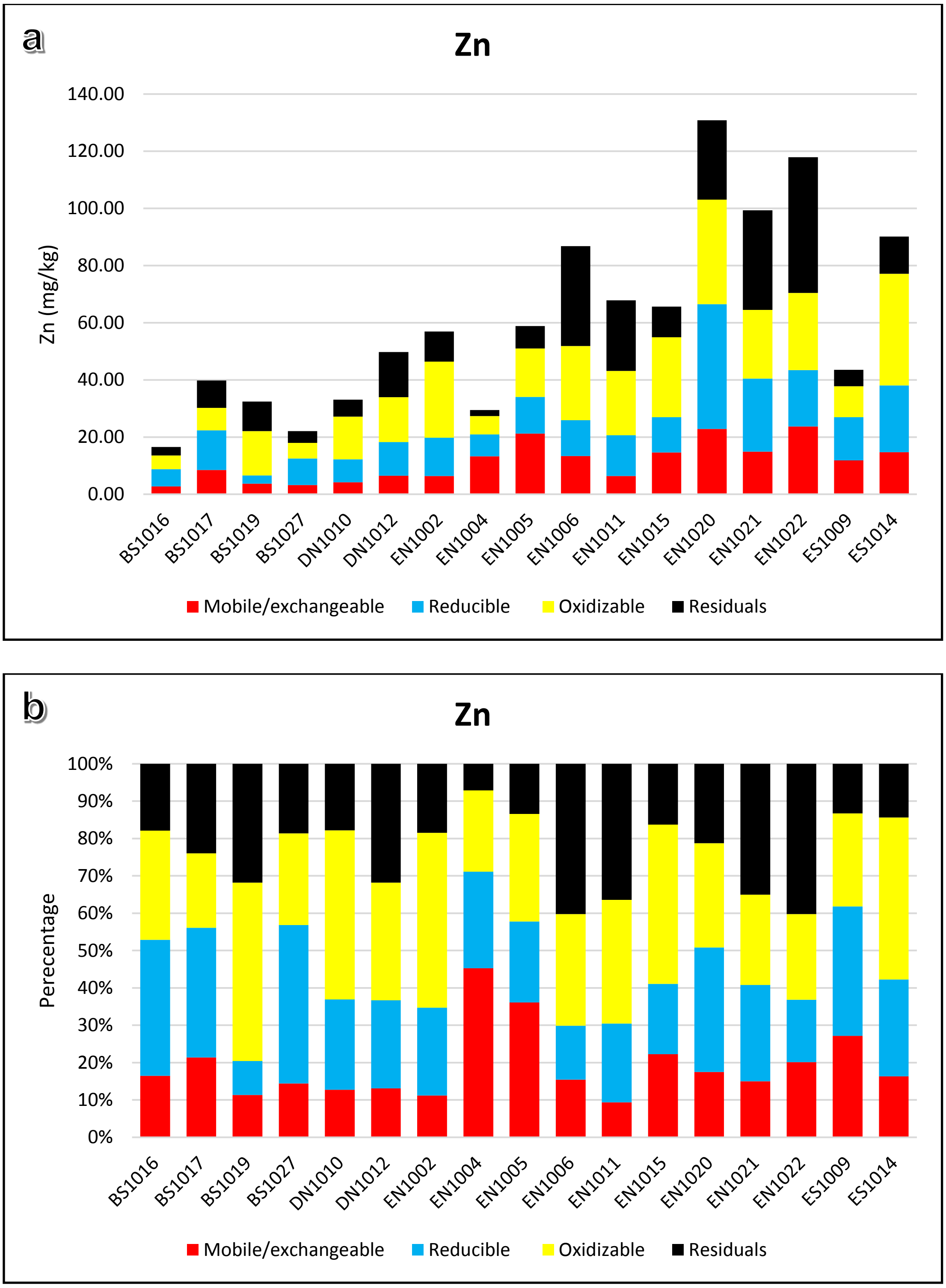


Fig. 39 (**a** Chemical fractionation of Zn (mg.km^{-1}), **b** Zn distribution (%) in the geochemical fractions)

4.3. Accumulation of toxic metals in biota samples (Determination by AAS):

The accumulation of toxic metals in spring biota samples were analysed in 15 sampling sites.

Crustacea amphipods (freshwater shrimps) were found in nine springs, in two of them, BS1027 and DN1010, only was found this specie. In most of the springs were found various feeding groups of species (Table 5). In EN1005, ES1008 an ES1014 predators predominated, and shredders and collectors in the other springs. The weight of biotic samples ranged from 0.01 to 0.19 g, in ES1014 was found caddisfly larvae (Trichoptera) in greater quantity (0.10g), this was the reason to analyse it separately.

Table 5. Macroinvertebrates in the springs sites

Feeding groups	Species
Shredders	Trichoptera (caddisfly larvae) amphipods (*grammarus* and *niphargus*) caddisfly larvae
Collectors/Filter feeders	Bivalvia (freshwater mussel) turbellaria (flatworms) Oligochaeta (segmented worm) stratiomyidae (soldier flies)
Predators	Athericidae (water snipe fly), ceratopogonidae (biting midges, midges larvae), bugs

Cu concentrations (Fig. 40) ranged from 4.86 mg.kg^{-1} at ES1014 - caddisfly to 53.52 mg.kg^{-1} at ES1009 – shrimps; ES1009 - shrimps and ES1014 - shrimps exceeded the EQS given by USEPA (31.6 mg Cu kg^{-1}). Pb concentrations (Fig. 41) ranged from close to 0 mg.kg^{-1} at EN1021 - shrimps to 6.93 mg.kg^{-1} at EN1002 - mix, however any biota sample exceeded the EQS given by USEPA (35.8 mg Pb kg^{-1}). Zn concentrations (Fig. 42) ranged from 46.82 mg.kg^{-1} at EN1002 - mix to 191.44 mg.kg^{-1} at BS1016 – mix; ES1014 - mix (142.82 mg.kg^{-1}), ES1014 - shrimps (122.57 mg.kg^{-1}), EN1004 - mix (171.40 mg.kg^{-1}) and EN1006 - mix (179.08 mg.kg^{-1}) exceeded the EQS given by USEPA (121 mg Zn kg^{-1}). Cd concentrations (Fig. 43) ranged from 0.01 mg.kg^{-1} at ES1009 - mix to 5.99 mg.kg^{-1} at EN1004 - mix; ES104 - caddisfly (2.07 mg.kg^{-1}), EN1005 - shrimps (2.33 mg.kg^{-1}) and mix from EN1021 (1.20 mg.kg^{-1}), EN1005 (1.30 mg.kg^{-1}), EN1011 (1.70 mg.kg^{-1}), ES1014 (2.14 mg.kg^{-1}), EN1001 (2.17 mg.kg^{-1}), DN1012 (2.94 mg.kg^{-1}), EN1005 (4.81 mg.kg^{-1}), and BS1016 (5.65 mg.kg^{-1}) exceeded the EQS given by USEPA (0.99 mg Cd kg^{-1}). Biota samples reflected lower Pb concentrations and did not overpass the EQS benchmarks, the highest Zn and Cd concentrations were found in BS1016 and EN1004, and they exceeded the EQS benchmarks.

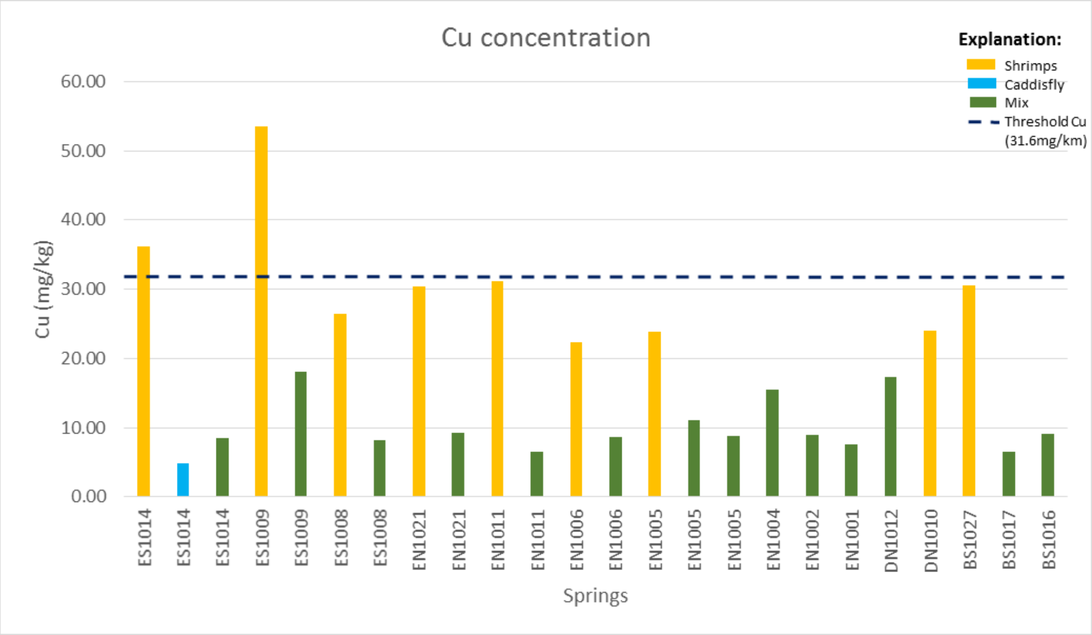


Fig. 40 Concentration of Cu in biota of sampling sites.

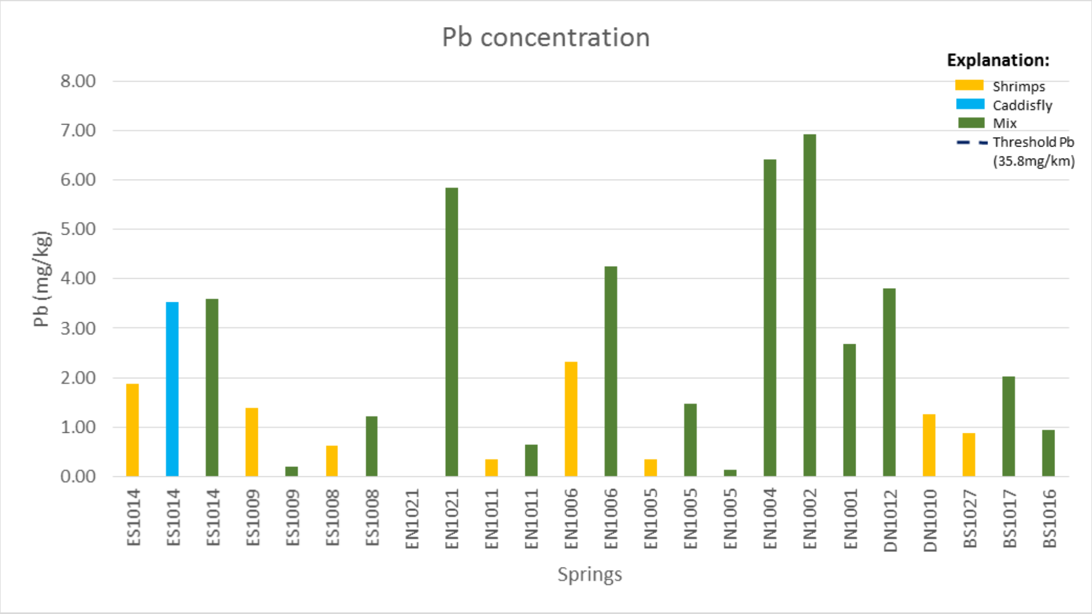


Fig. 41 Concentration of Pb in biota of sampling sites.

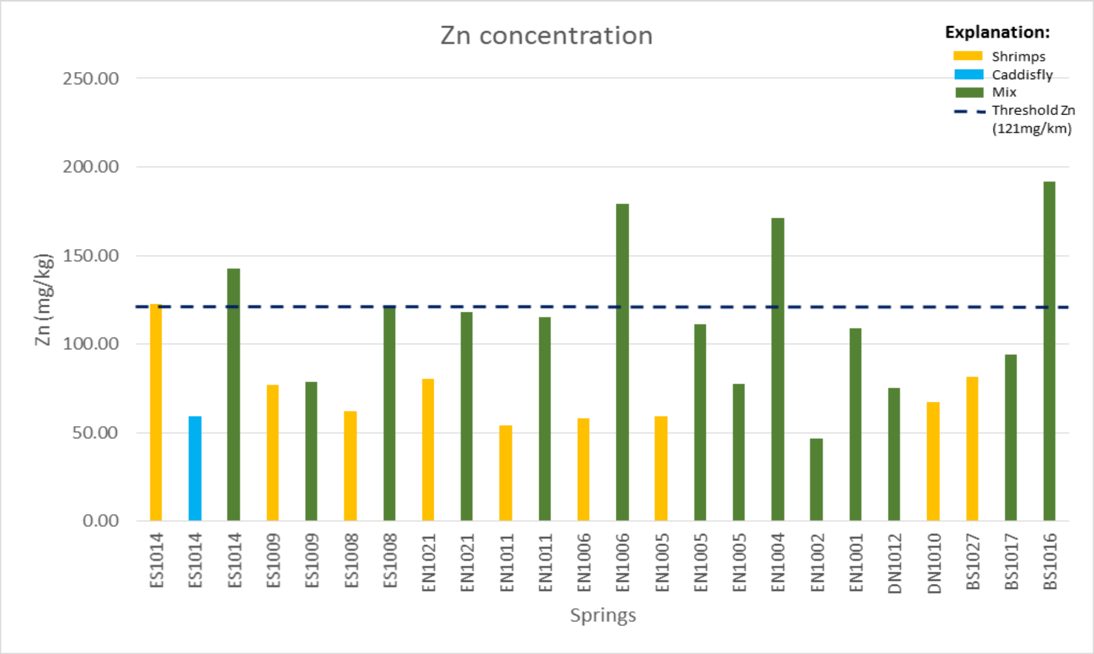


Fig. 42 Concentration of Zn in biota of sampling sites.

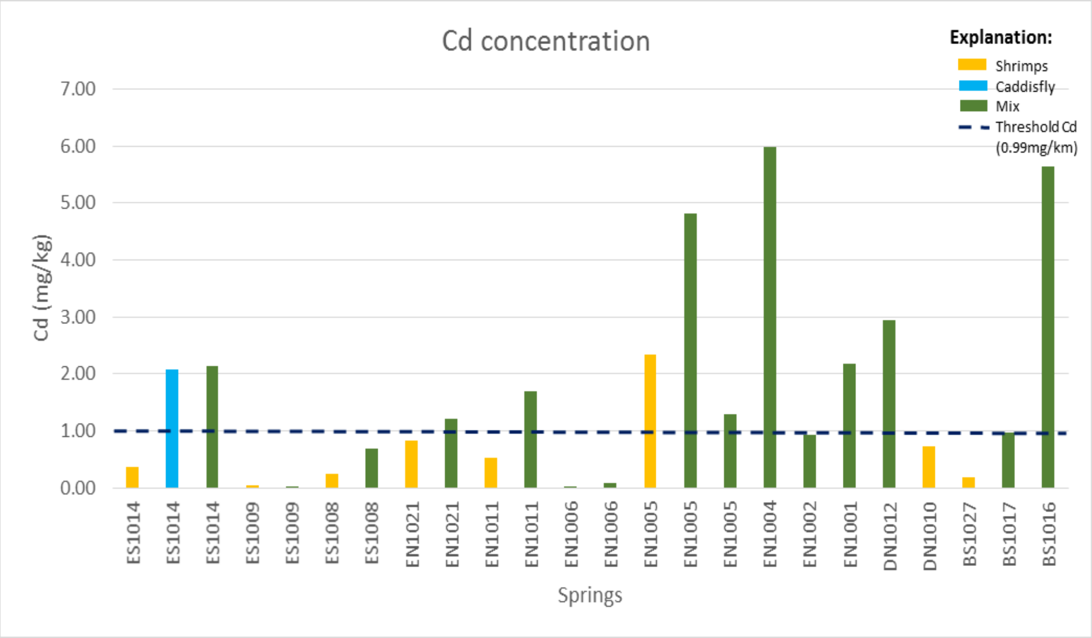


Fig. 43 Concentration of Cd in biota of sampling sites.

4.4. Hazard assessment:

The hazard assessment, performed using the indexes described above, showed interesting results. According to the distribution coefficient (Table 6), the metal most easily released from the sediment is Cu (log Kd 2.8 at BS1017 site). Differences in log Kd values among sampling sites for particular metals are seen as well. For instance, Zn and Cd are most available from sediments of BS1016, but Kd values for Zn are mostly >4. Higher Kd values for Pb (5.01 – 6.62) reflect lower availability, which means lower migration potential into liquid phase.

Table 6. Average distribution coefficient (log Kd) of studied sites

Log Kd	Cu	Zn	Cd	Pb
BS1016	3.97	3.53	3.54	5.96
BS1017	2.80	4.31	4.10	5.01
BS1019	NA	4.43	3.60	5.40
BS1027	NA	4.38	3.39	5.48
DN1012	4.27	4.24	3.63	5.89
EN1001	4.17	4.31	4.10	5.90
EN1002	3.79	4.28	3.48	6.09
EN1004	4.80	4.29	3.48	6.59
EN1005	5.58	4.77	4.69	5.89
EN1006	4.68	4.49	3.52	6.37
EN1011	5.21	5.53	4.88	6.50
EN1015	3.80	4.38	3.43	5.97
EN1021	4.78	4.10	4.51	6.14
EN1022	5.46	4.07	3.95	6.62
ES1008	3.27	4.12	3.63	5.34
ES1009	4.51	4.63	3.84	6.41
ES1014	3.88	4.76	4.87	5.88

NA – data not available (Cu concentration under detection limit – DL)

The hazard quotient (HQ – TEC) (Table 7) indicates dangerous concentrations of Pb in 47% of sediments, especially in sediment from EN1021 site, which also reflects slightly higher concentration of Cu, Zn and Cd among the sediments. The value of HQ – TEC >2 for Pb in this site probably originate in runoff from heavy traffic areas from the upper side. Cu can be evidence of either industrial waste water impacts or runoff from residential areas, where Cu materials are used for roofs components. Sediments from EN1021 site are slightly loaded by Cu (1.31) and Zn (0.69) and according to Clements et al. (2000), the HQs (ranging from 1- 2) indicate low environmental danger with no acute risks to the benthic community. According to MacDonald et al. (2000) a mean PEC quotient of 0.5 is a useful threshold for determining whether or not sediment samples are toxic (Burton, 2002). The hazard quotient (HQ – PEC) values (Table

7) of sediments reflect lower concentrations in general, except EN1021. The value of HQ – PEC > 0.5 for Pb in EN1021 indicates higher toxic concentration of Pb in sediments, this result is congruent with HQ – TEC value for sediments from EN1021. According to HQ, sediments of springs do not seem to be significantly loaded by toxic metals (with the exception of Pb in EN1021 and EN1004, where there are a high sediment load). The bioavailability for Cd, Cu, Pb and Zn metals was evaluated by the Mobility Factor and Risk Assessment Code (Table 8) based on the sequential extraction procedure (Nábělková & Komínková, 2012 ex. Nábělková, 2005; Tessier, 1979) performed on selected sediment samples.

Table 7. An average value of HQ (TEC, PEC) for metals concentrations in sediment and BSAF for Metals in studied sites

Springs	**Cu**			**Pb**			**Zn**			**Cd**		
	BSAF	**HQ – TEC**	**HQ – PEC**	**BSAF**	**HQ – TEC**	**HQ – PEC**	**BSAF**	**HQ – TEC**	**HQ – PEC**	**BSAF**	**HQ – TEC**	**HQ – PEC**
BS1016	3.78	0.08	0.02	0.07	0.38	0.11	16.89	0.09	0.02	81.89	0.07	0.01
BS1017	13.36	0.02	0.00	0.08	0.70	0.20	2.02	0.39	0.10	5.89	0.16	0.03
BS1027	NA	0.00	0.00	0.20	0.13	0.04	1.11	0.60	0.16	7.02	0.02	0.00
DN1010	4.69	0.16	0.03	0.03	**1.20**	0.34	1.62	0.34	0.09	32.64	0.02	0.00
DN1012	4.05	0.14	0.03	0.08	**1.31**	0.36	1.24	0.50	0.13	6.85	0.43	0.09
EN1001	1.50	0.16	0.03	0.09	0.83	0.23	1.79	0.50	0.13	8.65	0.25	0.05
EN1002	1.41	0.20	0.04	0.37	0.52	0.15	1.78	0.22	0.06	21.83	0.04	0.01
EN1004	2.70	0.18	0.04	0.11	**1.64**	0.46	4.61	0.31	0.08	23.54	0.26	0.05
EN1005	1.53	0.91	0.19	0.17	0.32	0.09	4.08	0.50	0.13	42.89	0.20	0.04
EN1006	3.89	0.25	0.05	0.19	0.98	0.27	3.33	0.59	0.15	0.87	0.10	0.02
EN1011	2.10	0.57	0.12	0.02	**1.33**	0.37	2.49	0.56	0.15	7.36	0.30	0.06
EN1021	0.96	**1.31**	0.28	0.07	**2.34**	**0.65**	2.37	0.69	0.18	2.08	0.98	0.20
ES1008	12.36	0.09	0.02	0.17	0.30	0.08	16.10	0.09	0.02	27.00	0.03	0.01
ES1009	7.59	0.30	0.06	0.04	**1.09**	0.30	5.42	0.24	0.06	1.74	0.03	0.01
ES1014	16.44	0.10	0.02	0.20	**1.28**	0.36	5.18	0.52	0.14	6.19	0.75	0.15

NA – data not available (Cu concentration under detection limit – DL)
Bold Font: TEC >1, PEC >0.5

A low bioavailability risk (RAC 2) was found for Pb and Cu in three studied sites, BS1019, EN1020 and EN1021, although these sites showed high bioavailability risk (RAC 4) for Cd, except for EN1021 sediment where the risk for Cd was very high (RAC 5). 47% of the sediments presents a RAC 2 for Pb. Zn reflects that 41% of sediments indicate a RAC 4 and also 41% a RAC 5, it means Zn has a very high bioavailabity risk among the sediments. About 59% of sediments present a high bioavailability risk (RAC 4) for Cu. Cd showed a very high bioavailability risk (RAC 5), about 59% of sediments. It can be assumed that a significantly higher contaminant concentration reflects a higher proportion in more bioavailable geochemical sediment fractions (exchangeable and carbonates). An intermediate bioavailability risk (RAC 3) was found for Cd, Zn and Pb in sediments from EN1006 site, and a very high bioavailability risk (RAC 5) was found for Cd, Zn and Cu in sediments from BS1017 and EN1004 sites.

Table 8. The MF and RAC Hazards of toxic metals in Sediments

Springs	Cd		Zn		Cu		Pb	
	MF	RAC	MF	RAC	MF	RAC	MF	RAC
BS1016	42.88	4	52.90	5	45.58	4	5.32	2
BS1017	78.23	**5**	56.14	**5**	51.49	**5**	40.53	4
BS1019	31.60	4	20.41	3	7.02	2	9.26	2
BS1027	11.45	3	56.81	5	46.64	4	3.28	2
DN1010	83.30	5	36.89	4	37.56	4	33.98	4
DN1012	78.99	5	36.68	4	22.79	3	50.03	5
EN1002	59.04	5	34.73	4	31.43	4	67.95	5
EN1004	92.06	**5**	71.10	**5**	79.31	**5**	23.21	3
EN1005	26.13	3	57.80	5	37.37	4	37.02	4
EN1006	29.29	3	29.84	3	32.94	4	22.18	3
EN1011	59.19	5	30.49	3	23.77	3	7.54	2
EN1015	72.70	5	41.09	4	23.17	3	50.48	5
EN1020	47.98	4	50.85	5	7.28	2	3.65	2
EN1021	66.34	5	40.80	4	5.30	2	4.00	2
EN1022	91.24	5	36.85	4	73.60	5	4.05	2
ES1009	19.54	3	61.83	5	49.69	4	34.29	4
ES1014	82.57	5	42.26	4	18.43	3	2.54	2

Bold Font: RAC 5 for Cd, Zn and Cu in BS1017 and EN1004

According to the MF and RAC, Cd is the most available and hazardous in most sites, and is even a very high risk (RAC 5) for BS1017 and EN1004 sites. The higher bioavailability of metals in the BS1017 and EN1004 sites is clear from Table 6. The higher bioavailability of metals in the EN1004 site may be caused by high contamination (particularly in the case of Cd), because of its proximity to residential areas, and during the first few minutes of rain the pH of water can decrease due to pollution washed out from the air of urban areas, consequently leading to the remobilization of metals from sediments (Nábělková & Komínková, 2012 ex. Nábělková, 2005; Stumm & Morgan, 1996); while the BS1017 site, is far from the industrial area, consequently the concentration of Cd, Cu, and Pb are very low, except Zn (39.79 $mg.kg^{-1}$), however It does not overpass the EQS.

Average values of the Biota Sediment Accumulation Factor (Table 5) more or less confirm the results of MF and RAC. Cd in all springs accumulates in organisms to high levels. Organisms from BS1016 show significantly elevated accumulations of toxic metals, especially Cd and Zn.

4.5. Impact assessment of geological background on metal occurrence:

To assess the geological background impact on metal occurrence, we considered a means to differentiate between the metals weakly associated with the sediment and the metals that are strongly bound to the sediment and geological background. Weakly associated metals include metals in the mobile-exchangeable fractions and reducible fractions. Mobile and exchangeable metal ions are ions that are released most readily into the environment (Filgueras et al., 2002). Mobility factor MF (%) (Table 9) was used to express the metals weakly associated with the sediment. The metals strongly bound in the sediment include the metals leached in the residual fractions, which is incorporated into crystalline mineral lattices, and refractory unoxidized organic matter as undissolved amorphous iron oxide (Wijaya et al., 2016). The percentages of Residual fractions, obtained from sequential extraction procedure, were used.

Table 9. The Mobility factor (MF %) and the concentration in Residual fraction (RFr %) of toxic metals in Sediments

Springs	Cd		Zn		Cu		Pb	
	MF	RFr	MF	RFr	MF	RFr	MF	RFr
BS1016	42.88	NA	52.90	17.90	45.58	6.83	6.28	17.41
BS1017	78.23	NA	56.14	23.98	51.49	14.89	40.53	6.82
BS1019	31.60	11.99	20.41	31.79	7.02	57.44	9.26	67.94
BS1027	11.45	72.40	56.81	18.61	46.64	16.17	3.28	91.96
DN1010	83.30	NA	36.89	17.83	37.56	12.16	33.98	13.62
DN1012	78.99	2.92	36.68	31.79	22.79	10.90	50.03	35.85
EN1002	59.04	14.61	34.73	18.48	31.43	8.19	67.95	15.12
EN1004	92.06	3.97	71.10	7.09	79.31	7.13	23.21	73.82
EN1005	26.13	8.11	57.80	13.41	37.37	31.32	37.02	6.06
EN1006	29.29	31.58	29.84	40.25	32.94	31.44	22.18	32.78
EN1011	59.19	13.16	30.49	36.41	23.77	27.77	7.54	44.77
EN1015	72.70	13.51	41.09	16.26	23.17	9.10	50.48	27.47
EN1020	47.98	21.73	50.85	21.27	7.28	28.36	3.65	62.34
EN1021	66.34	9.89	40.80	35.03	5.30	17.66	4.00	66.48
EN1022	91.24	3.68	36.85	40.22	73.60	7.66	4.05	51.50
ES1009	19.54	40.23	61.83	13.26	49.69	16.81	34.29	38.05
ES1014	82.57	5.17	42.26	14.40	18.43	0.00	2.54	41.34

NA – data not available (Cd concentration under detection limit – DL)

Pb concentrations were released in residual fraction (RFr) (Fig. 7),and were reltively higher tan mobility factor (MF). Pb concentrations was up to 91.96% at BS1027 site. Cu concentrations are slightly higher in MF than in RFr among the sediments, except sediments from BS1019 (57%), EN1020 (28%) and EN1021(18%) are bound to RFr. Higher Zn concentrations were retained in MF than RFr. Zn concentrations ranged fom 20.41% at BS1019 site to 71.10% at site EN1004. Cd concentrations was up to 91.24% at EN1022 site. The most sediments showed high Cd concentrations in MF; which indicates high potential bioavailability an mobility of Cd; except BS1027, where 72.40% was retained in RFr and 11.45% in MF, and ES1009, where 40.23% was retained in RFr and 19.54% in MF. Pb (91.96%) and Cd (72.40%) concentrations of sediments from BS1027 site were strongly bound to residual fraction – RFr, while Zn (56.81%) and Cu (46.64%) concentrations from this site were found more available in MF. Pb concentrationiis and Cd within the residual fraction mainly originated from the parent materials of the sediment (Wijaya et al., 2016).

4.6. Statistical analyses

Toxic metals concentration in pseudototal fractions

Box plot charts for toxic metals concentration from pseudototal fractions, were generated for three parameters (geology type, spring type, pseudototal fractions) to enable easy comparison.

Toxic metal concentrations (Fig. 44) did not show significantly variations in the toxic metals concentration among the pseudototal fractions (<0.061mm, 0.206-0.061mm, 0.609-0.206mm, and >0.609mm); except Cu and Zn, those showed slightly higher concentrations in <0.061mm fraction. For this analyses, Cu concentration (629.26 mg/kg) in 0.206-0.061mm fraction from EN1021; and Cu concentration (325.05 – 606.50 mg/kg) and Cd concentration (325.05 – 606.50 mg/kg) in all pseudototal fractions from EN1022 were excluded, since they had extremely high concentrations. Cu concentration varied from very low (0.5 mg/kg) to relatively high (70 mg/kg) in <0.061mm fraction. Zn concentrations in <0.061mm fraction (31 – 158 mg/kg) were relatively high; however, 0.206-0.061mm fraction (8 – 157 mg/kg), 0.609-0.206mm fraction (6 – 136 mg/kg) and >0.609mm fraction (11 – 135 mg/kg) also indicated high Zn concentration, on the other hand the lowest Zn concentration were found in 0.609-0.206mm fraction. Cd concentrations showed the lowest values in <0.061mm fraction(0.01 – 0.844 mg/kg) and the highest in >0.609mm fraction (0.02 – 0.972 mg/kg); although, Cd concentrations did not vary significantly. Pb concentrations reflected slightly variation among the pseudototal fractions, the lowest Pb concentration was found in 0.609-0.206mm fraction (0 – 70 mg/kg) and the highest concentration was found in 0.206-0.061mm fraction (5 – 87 mg/kg).

Acconding the results of correlation between toxic metal concentrations (mg/kg) and geology type (Table 10), the highest concentrations for Cd (0.97 mg/kg) and Zn (157.1 mg/kg) were found in CAM3, and the lowest concentrations for these two metals were very variable. The lowest Cd concentrations (0.01 mg/kg) were found in CRA 1. The lowest Zn concentrations (5.54 mg/kg) were found in CRA 2. The highest Cu concentrations (70.88 mg/kg) were found in CRA2. The lowest Cu concentrations (close to 0 mg/kg) were found in CRA 1 and CRA 2. The highest Pb concentrations (87.02 mg/kg) were found in CAM 3 . The lowest Pb concentrations (close to 0 mg/kg) were found in CRA 1 (Fig. 45).

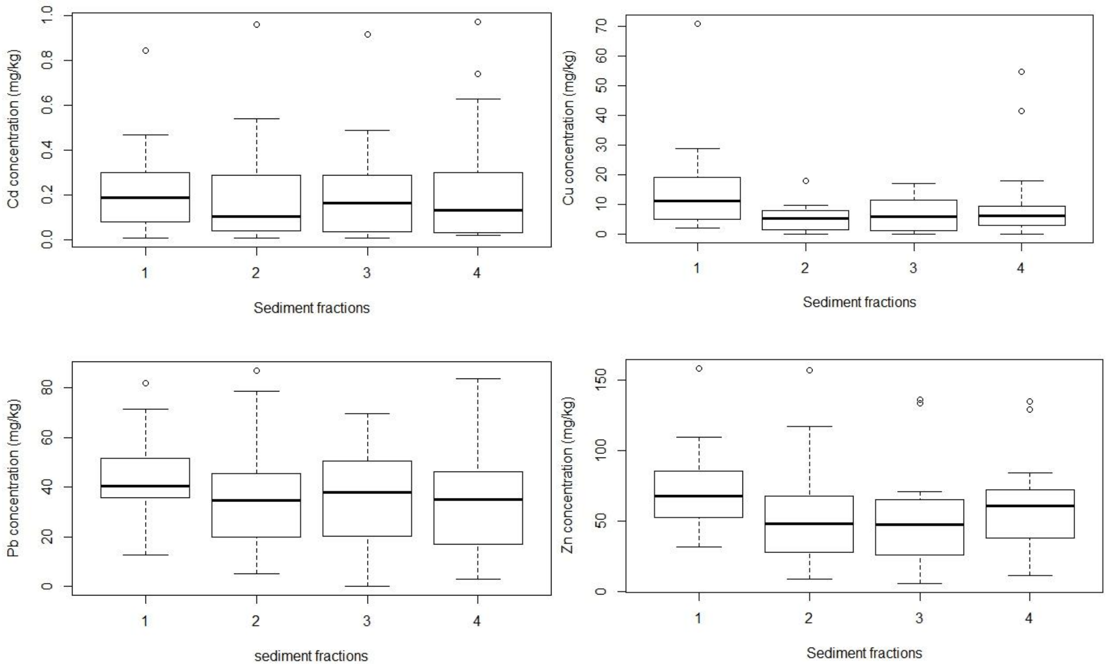


Fig. 44 Toxic metals concentration (mg/kg) in pseudototal fractions.

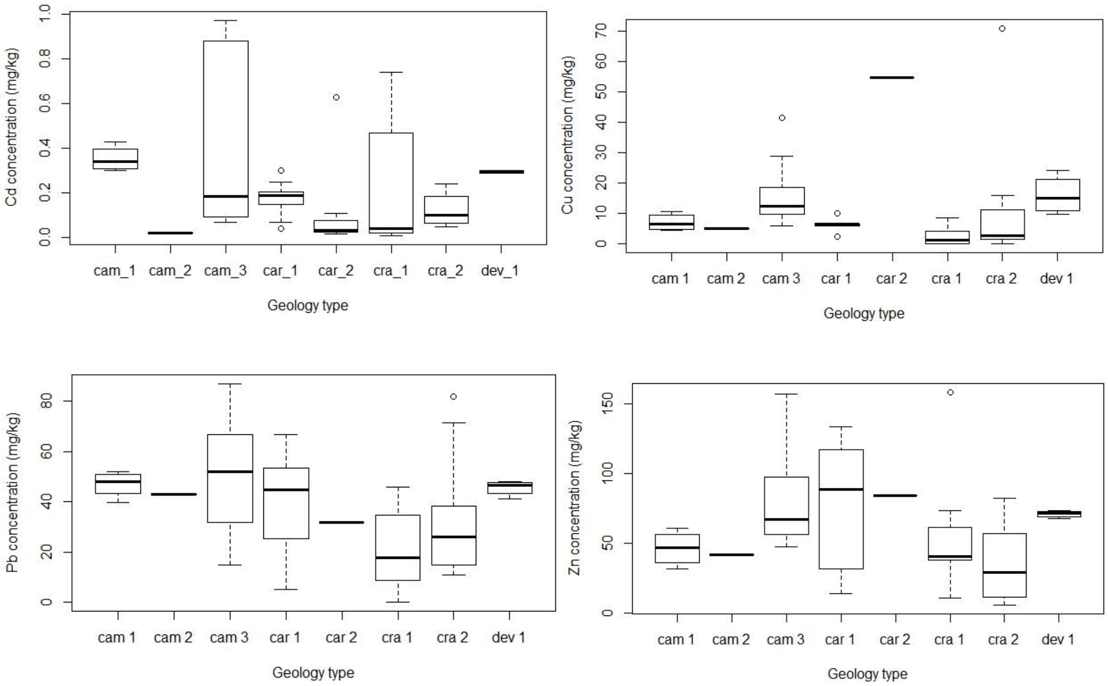


Fig. 45 Toxic metals concentration (mg/kg) in pseudototal fractions between the different geology types.

Table 10. Description of geology types

Geology type	Description
CAR 1	Carboniferous (Early Permian), biotite-monzogranites
CAR 2	Carboniferous (Early Permian), muscovite-biotite monzogranites
CRA 1	Cretaceous, quartz sandstones
CRA 2	Cretaceous, sandstones
CAM 1	Cambrian, augen gneisses
CAM 2	Cambrian, limestones
CAM 3	Cambrian, sericite quartzites
DEV 1	Devonian, limestones

The results of correlation between toxic metal concentrations (mg/kg) and spring type reflected that the highest concentration of Cu (70.88 mg/kg), Pb (87.02 mg/kg), Zn (158.31 mg/kg) were found in rheocrene, and the highest concentration of Cd was found in helocrene. The lowest concentration of Cu (close to 0 mg/kg), Pb (close to 0 mg/kg), Cd (0.01 mg/kg) were found in rheocrene, and the lowest concentration of Zn (5.54 mg/kg) was found in limnocrene (Fig. 46).

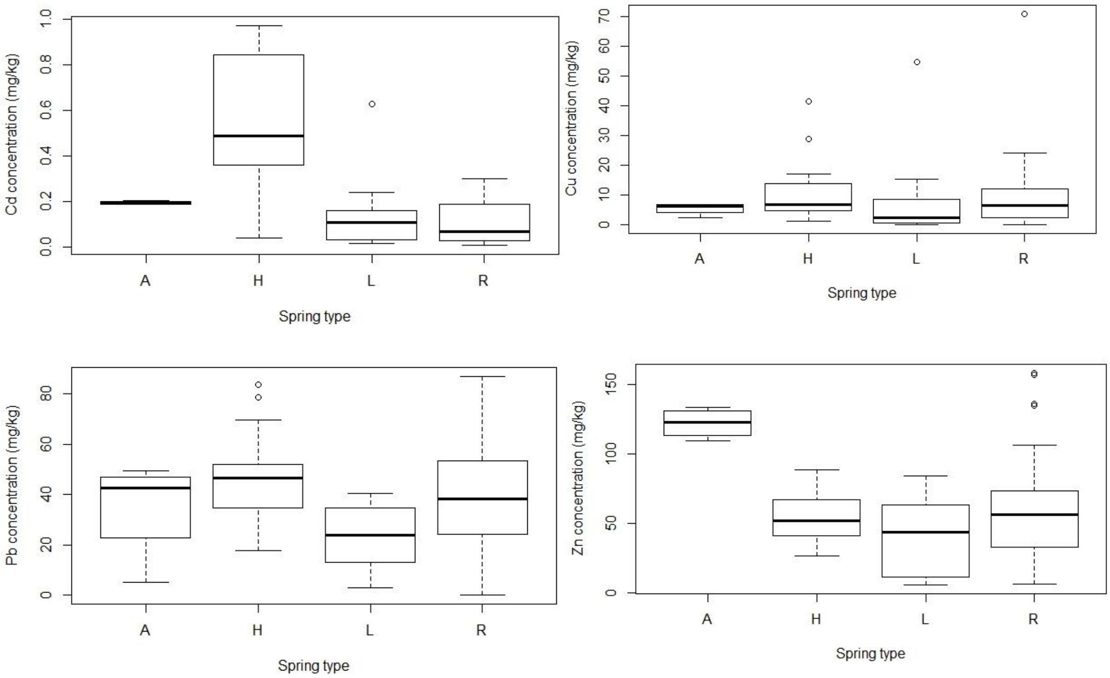


Fig. 46 Toxic metals concentration (mg/kg) in pseudototal fraction between the different spring types.

Toxic metals concentration in geochemical fractions

Box plot charts for toxic metals concentration from geochemical fractions, were generated for three parameters (geology type, spring type, and geochemical fractions) to enable easy comparison.

Cu concentrations of all geochemical fractions (exchangeable, reducible, oxidizable, and residuals) from EN1022 (27.25 – 188.92 mg/kg), oxidizable fraction of EN1021 (39.48 mg/kg) and oxidizable fraction of EN1020 (28.48 mg/kg) were not used, due to their high concentrations. Toxic metal concentrations reflected slightly variations. Very low concentration of Pb and Cu were released in the exchangeable fraction, which indicated that these metals do not represent detrimental risk for aquatic environment. However, Pb concentrations in the reducible fraction varied from (close to 0 mg/kg) to slightly high (20 mg/kg). Zn concentrations were relatively retained more in oxidizable, and residuals fractions, although Zn concentrations in the exchangeable fraction varied from (30 mg/kg) to relatively high (110 mg/kg). Cd concentrations do not show significantly variation, the lowest Cd concentration were found in the reducible fraction, although Cd concentrations in the exchangeable fraction were slightly higher. In this analyses the exchangeable fraction (2.73 mg/kg) and reducible fraction (2.28 mg/kg) of EN1022 was excluded, due to their extremely high Cd concentrations (Fig. 47).

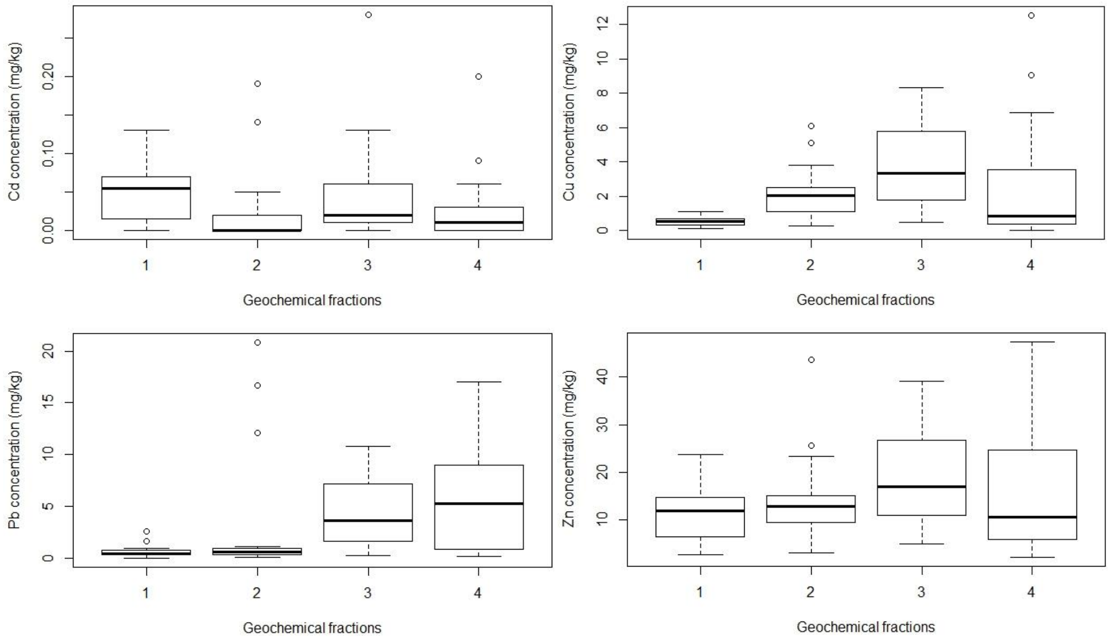


Fig. 47 Toxic metals concentration (mg/kg) in geochemical fractions

According the results of correlation between toxic metal concentrations (mg/kg) and geology type, the highest concentrations for Cd 0.28 and Zn 47.43 were found in CAM3. The lowest Cd concentrations (close to 0 mg/kg) were found in CRA 1, CRA2, CAR 1, CAM 2 and CAM 3. The lowest Zn concentrations (2.09 mg/kg) were found in CAR 1. The highest Cu concentrations (12.55 mg/kg) were found in CAR 2. The lowest Cu concentrations (close to 0 mg/kg) were found in CRA 1. The highest Pb concentrations (20.84 mg/kg) were found in CAR 1. The lowest Pb concentrations (close to 0 mg/kg) were found in CRA 2 (Fig. 48).

According to results, the highest concentration of Cd (0.28 mg/kg), Zn (47.43 mg/kg) were found in rheocrene; the highest concentration of Cu (12.55 mg/kg) was found in limnocrene and the highest concentration of Pb (20.84 mg/kg) was found in helocrene. The lowest concentration of Zn (2.09 mg/kg), Pb (close to 0 mg/kg) were found in rheocrene, the lowest concentration Cd (close to 0 mg/kg) was found in rheocrene, limnocrene and helocrene, and the lowest concentration Cu (close to 0 mg/kg) was found in helocrene (Fig. 49).

The results of covariance analyses of toxic metals concentration using linear models, indicated a positive correlation between the toxic metals concentration (Fig. 50).

The most positive correlation between the model of covariance (Table 11) was found in model 2, between Cd and Cu (R-squared: 0.9288); model 1 also reflected a good correlation (R-squared: 0.4201) (Fig. 51). There was a positive correlation between Pb concentration in sediments and biota (crustaceans (shrimps) and mix), specially with crustaceans (R-squared: 0.2083), while Cu concentration in sediments and biota (shrimps) showed the most negative correlation (R-squared: 6.817e-05).

Table 11. Description of covariance model

Models	Metals correlation
Model 1	Cd ~ Zn
Model 2	Cd ~ Cu
Model 3	Cd ~ Pb
Model 4	Cu ~ Zn
Model 5	Cu ~ Pb
Model 6	Pb ~ Zn

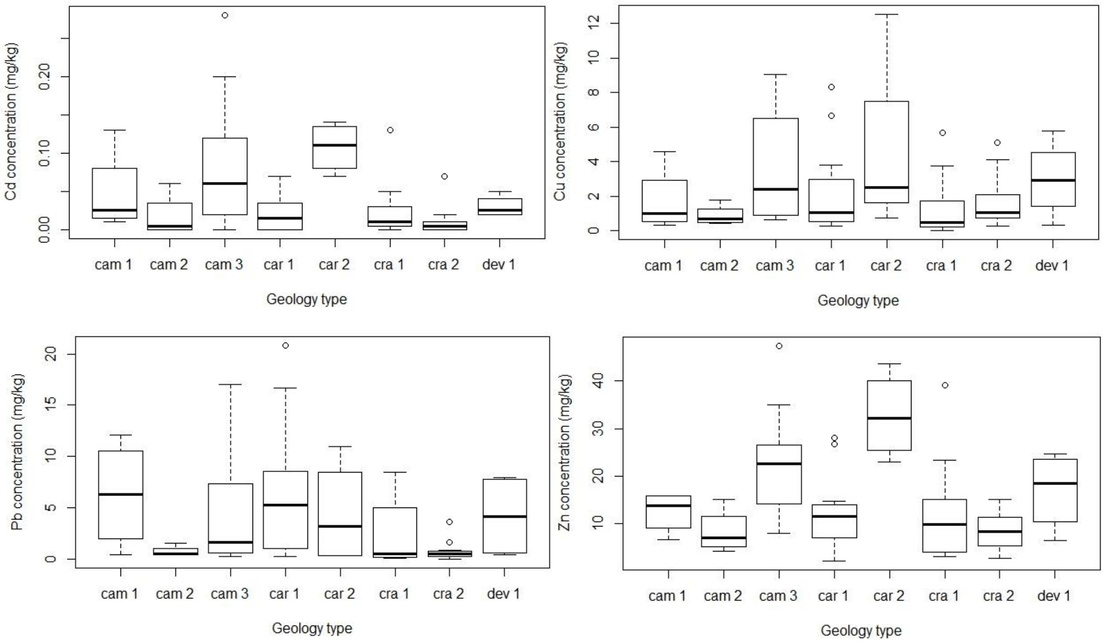


Fig. 48 Toxic metals concentration (mg/kg) in geochemical fractions between the different geology types.

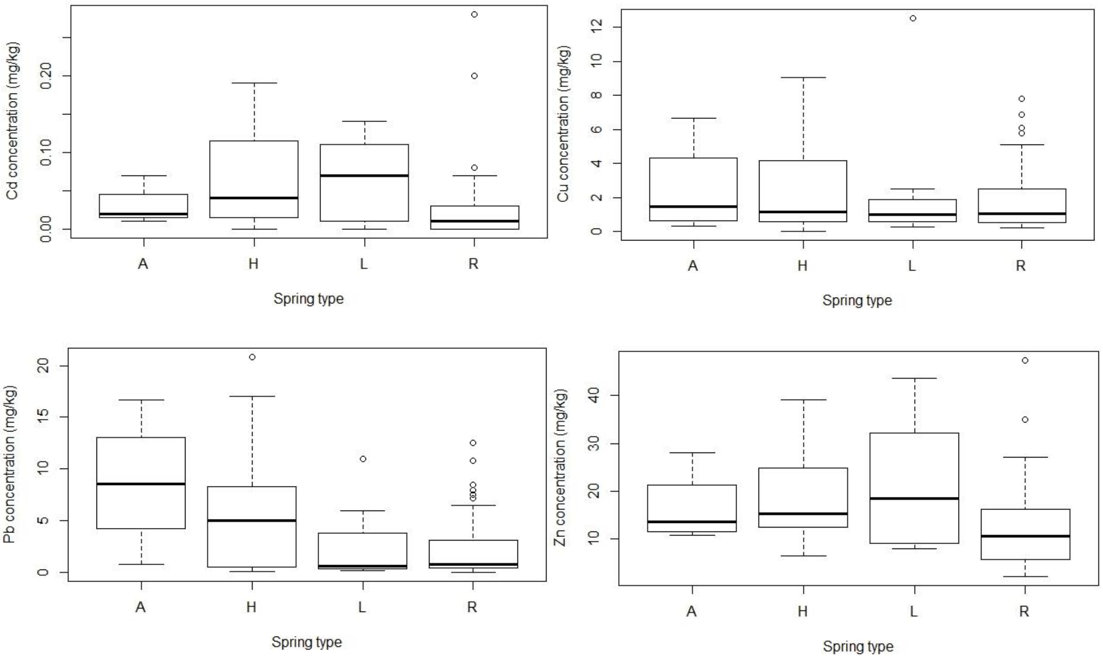


Fig. 49 Toxic metals concentration (mg/kg) in geochemical fractions between the different spring types.

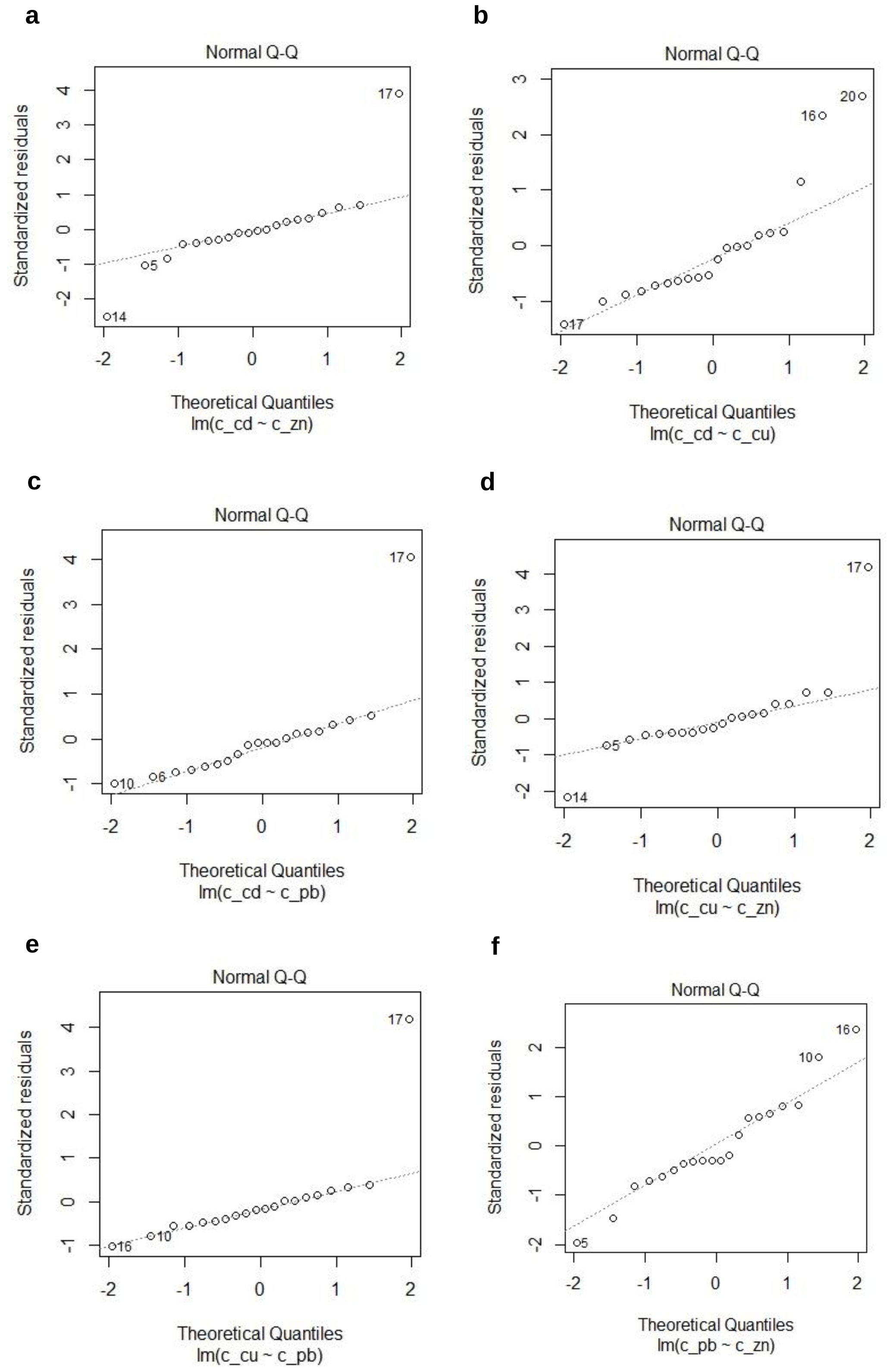


Fig. 50 Analyses of covariance between toxic metals concentration (mg/kg) using linear models (LM) (**a** model 1 Cd~Zn, **b** model 2 Cd~Cu, **c** model 3 Cd~Pb, **d** model 4 Cu~Zn, **e** model 5 Cu~Pb, **f** model 6 Pb~Zn)

V. Discussion:

The appearance of a spring and its macroinvertebrate composition depend on many factors, each of which varies considerably. (Von Fumetti, 2008). All springs we investigated in this study are cold-temperate springs, according to Alfaro and Wallace (1994), with an average temperature of 7-12 °C throughout the year (Table 1). In some springs during spring season the temperature increased to 14.1°C.

Springs characterized by a low level of temporal variability of hydrological and hydrochemical conditions compared with other freshwater sources. Thus, the species inhabiting spring ecosystems are often strictly associated with these ecosystems (Glazier, 1991; Hahn, 2000; Schindler, 2004; Gerecke et al., 2009; Cantonati et al., 2012). According to Von Fumetti and Nagel (2012) Springs can also be strongly influenced by disturbances such as drought or heavy rainfall. The research area is located in one of the most moisture regions of the Czech Republic, and the results of basic water parameters of all springs were not constant, pH, conductivity, temperature and OD relatively decreased in the transition from spring to autumn; acid pH predominated in springs during the autumn season. Champion and Starks (2001) affirmed that the pH of spring water typically varies between 7 and 8, indicating that the groundwater is at or near chemical equilibrium with limestone in the aquifer. As pH decreases, the concentration of metal may increase because higher acidity increases their ability to be dissolved from sediments into the water (Anonymous, 1996).

The mobility and bioavailability of metals bound to sediments depend on multiple factors, with sediment characteristics and the physical–chemical form of the metal being the key factors (Prokop et al., 2002). It is suggested the sediment sources influenced the mixture and composition, character, and chemistry of the metals (Wijaya et al., 2016). The oxidation of sulfides and organic matter may also generate if the buffer capacity of the receiving environment is not sufficient, acidic conditions, which may provoke increased mobility of some metals. However, the mobile fraction does not necessarily correspond to the bioavailable fraction (Prokop et al., 2002).

Since total metal concentration using the standard quality guidelines – SQG cannot provide the proper information about the availability of the toxic metal, and in concordance with Prokop et al. (2002), mobile and bioavailable fractions were considered as important factors in the accumulation of toxic metals in the sediment and biota of springs. The high mobility factor – MF and biota sediment accumulation factor – BSAF values in this study have been interpreted as symptoms of relatively high lability and biological availability of toxic metals. The following order of bioavailability was found with the toxic metals Cd > Zn > Cu > Pb.

Metals associated with oxidizable fractions are assumed to remain in sediments for longer periods and are possibly mobilized by decomposition processes (Filgueiras et al., 2002). Ratuzny et al. (2009) reported that oxidizable metals may be associated with a stable and high molecular weight humic substance that releases metals in a slow manner (Wijaya et al, 2016). In this study, copper was associated with organic matter, and its mobility was controlled by the concentration and degradation of the organic fraction. Copper is an essential metal for most organisms (Clarkson et al., 1991), although, beyond certain threshold levels (Prato et al., 2013) copper ions are extremely toxic for aquatic organisms and pose a threat to many aquatic organisms when available in excess (De Martinez et al., 2011). Copper ions can enter freshwater systems for example by runoff caused by strong rainfalls, pollution of nearby springs is likely (Rychla et al., 2015). Pollution of springs can occur through entry of contaminated organic matter, run-off of contaminants directly into the water or via the groundwater or via soil erosion (Schmidlin, 2015).

The lead was found to be the least labile, with up to 92% associated with the residual fraction of the sediments, although more than 50% of springs sediments exceeded the EQS of USEPA. The low Pb concentration in the exchangeable fraction was reflected in the biota, where the concentrations ranged from close to 0 – 6.93 mg.kg-1.

Most of Cd was strongly retained in the exchangeable fraction. The high solubility of Cd is mainly affected by the pH of the sediments and soils (Ratuzny et al., 2009). A decrease in pH (e.g., an effect of acid rain) may cause a release of metals from complexes and from solid matter surface by increased competition for sorption sites by the H^+ ion (Prokop et al., 2002).

Springs are important habitats for numerous specialized and rare species (Lindegaard et al., 1998) which are necessary for the entire ecotone to function sustainably. Spring species are adapted to the relatively stable environmental conditions in springs (e.g. Danks & Williams, 1991; Ferrington, 1995), many of them being cold-stenothermal (e.g. Fischer et al., 1998) (Schmidlin, 2015)

Gammarus fossarum (Crustacea; Amphipoda) is a relatively robust representative of the macrozoobenthos of springs. Since it inhabits springs and spring brooks abundantly, mainly in the central and eastern mountainous areas of Europe (Janetzky, 1994; Pöckl et al., 2003), it can be considered a suitable organism for assessing possible impacts of global change on species inhabiting springs. For all types of springs, specific inhabited taxa of Crustaceans (freshwater shrimps) was found, with the exception of two helocrene springs DN1012, EN1002, and limnocrene spring BS1017. A statistically significant correlation between this habitat types and species diversity cannot be described.

Graening and Brown (2000) made a research in springs cave and the results showed that isopods, crayfish, cavefish, and sediment have substantially higher concentrations of toxic metals than water samples. However, there was no significant trend of increasing metal concentration in the biota with increasing trophic level; while, Rychla et al. (2015) stated that the high concentrations of toxic metals (Zn, Pb, Cu, Cr, and Cd), as well as the total levels of iron and of manganese, did not influence the presence of *C. irrorata* (Trichoptera). The species was observed in certain springs with the highest seasonal (extreme) values of these metals, maximally reaching 0.23 mg.L^{-1} Zn, 0.13 mg.L^{-1} Pb, 0.043 mg.L^{-1} Cu, 0.012 mg.L^{-1} Cr, 0.005 mg.L^{-1} Cd.

Nevertheless, the accumulation of toxic metals in biota of Lužické mountains springs demonstrated that the pseudototal metal accumulation in sediments did is not enough for the assessment of the availability of the toxic metals. Concentrations of Cd and Zn in sediments of springs were mostly released in the exchangeable fraction, and only two springs overpassed the Cd and Zn pseudototal concentrations of the EQS of USEPA. Concentrations of Cd and Zn in biota of springs were significantly elevated than in sediments, six springs overpassed the Zn concentrations and eight springs overpassed the Cd concentrations of the EQS of USEPA. In this sense, exhaustive analyses of toxic metals (Cd, Zn, Cu and Pb) concentration in the geochemical fractions of spring sediments are needed to the assessment of the risk of the accumulation of these metals in spring biota.

VI. Conclusion:

Toxic metals accumulation in sediments and biota of springs of the research are very variable. Cd is the most available and hazardous in most springs sites, 59 of spring displayed a very high risk (RAC 5), it means the Cd concentrations in sediments were retained more than 50% in the exchangeable and reducible fractions. Pseudototal fraction reflected very low concentrations of Cd, only two springs, EN1006 (1.59 mg.kg^{-1}) and EN1022 (3.56 mg.kg^{-1}), exceeded the EQS of USEPA and he sum of Cd concentration in the geochemical fractions indicated that only EN1022 (5.49 mg.kg^{-1}) exceeded the EQS of USEPA; while the accumulation of Cd in biotic springs were very high, eight from fifteen springs exceeded the EQS of USEPA. Very low concentration of Pb and Cu were released in the exchangeable fraction. The most Pb is mainly associated to the residual fraction; although Pb are more than their background values in almost half of the sampling sites. The accumulation of Pb in biotic springs were insignificantly, any springs overpass the benchmarks and also the range (from close to 0 to 6.93 mg.kg^{-1}) was very low. It means this toxic metal do not represent a hazard for springs and the aquatic environment.

Statistical analysis applied to the correlation of geology type, spring type and toxic metals concentration in pseudototal fraction and geochemical fraction (Cd, Zn, Cu, and Pb) information demonstrates that the according to the geology type, the most occurrence of toxic metals is in CAM 3 (Cambrian, sericite quartzites), and according to spring type is in rheocrene.

The EN1022 spring was the most contaminated with toxic metals Zn, Cu, Pb and Cd, specially Cu which can contaminate spring fauna and the vegetation in the surrounding. Results from pseudototal concentration reflected that more than 50% of springs contained high concentrations of Pb, that exceeded EQS of USEPA; although, most of this concentration was retained in residual fraction, organisms, since Pb is bound to organic matter, sulphides, or mineral fractions in spring. The metal most easily released from the sediment is Cu (log Kd 2.8 at BS1017 site), Zn and Cd are most available from sediments of BS1016; however, toxic metal metals concentration in these springs displayed the lowest values among springs sites, besides Zn and Cd concentrations in pseudotal fraction and in the geochemical fraction of BS1016 were found very low, the Zn (191.44 mg.kg^{-1}) and Cd (5.65 mg.kg^{-1}) in macroinvertebrates of this spring, were found in very high concentrations. Cd concentration in most of sediments was strongly retained in the exchangeable fraction. Spring biota was strongly affected by toxic metal accumulation especially in Cd, eight springs exceeded EQS of USEPA for Cd concentration, these concentrations ranged from 1.20 at EN1021 to 5.99 at EN1004; it can cause significant and irreversible damage to aquatic ecosystems. Therefore, it is necessary to initiate measures and monitoring to get a better understanding of the source of these pollutants.

VII. References:

- Aiju L., Yanchun G., Honghai W., Gao Peiling G., 2012. An Assessment of Heavy Metals Contamination in Xiaofu River Sediments through Chemical Speciation Study. International Journal of Earth Sciences 5(5): 1235-1240.
- Akan J.C., Abdurrahman F.I., Sodipo O.A., Ochanya A.E., Askira Y.K., 2010. Heavy metals in sediments from River Ngada, Maiduguri Metropolis, Borno State,Nigeria. J Environ Chem EcoToxicol 2: 131-140.
- Alfaro C, Wallace M (1994) Origin and classification of springs and historical review with current applications. Environ Geol 24:112–124
- Allan, D. J. (1995). Stream Ecology. Structure and function of running waters, First edition. Chapman & Hall, London.
- Anonymous, 1996. https://dnr.mo.gov/env/esp/waterquality-parameters.htm
- Anonymous, 2001. Water quality and macroinvertebrates. Water facts: Water and river commission. Second Edition. Source available in:
- Apha – American Public Health Association. 1995. Standard Methods for the Examination for Water and Wastewater (19th edition). Byrd Prepess Springfield, Washington.
- Barnthouse LW, DeAngelis DL, Gardner RH, Ońeill RV, Suter GV, Vaughan DS, 1982. Methodology for Environmental Risk Analysis. ORNL/TM-8167. Oak Ridge.
- Barquín J., Scarsbrook M.R., 2007. Management and conservation strategies for coldwater springs. Aquat Conserv Mar Freshwater Ecosys. doi:10.1002/aqc.884:
- Barquín, Death, 2009. Physical and chemical differences in karst springs of Cantabria, northern Spain: do invertebrate communities correspond?. Aquat Ecol 43:445–455. DOI 10.1007/s10452-008-9170-2
- Beyer H., 1932. Die Tierwelt der Quellen und Bäche des Baumberggebietes. Abhandlungen aus dem Westfälischen Provinzial-Museum für Naturkunde, 3, 9-189.
- Bianchini A., Bowles K.C., 2002. Metal sulfides in oxygenated aquatic systems: implications for the biotic ligand model. Comparative Biochemistry and Physiology Part C, 133: 51-64
- Blackwood M.A., Hall S.M., Ferrington L.C., 1995. Emergence of Chironomidae from Springs in the Central High Plains Region of the United States. Journal of the Kansas Entomological Society 68(2) suppl.: 132-151.
- Bonnail E., Sarmiento A.M., DelValls T.A., Nieto J.M., Riba I.. 2016. Assessment of metal contamination, bioavailability, toxicity and bioaccumulation in extreme metallic environments (Iberian Pyrite Belt) using Corbicula flumin. Sci Total Environ; 544: 1031–1044. doi:10.1016/j.scitotenv.2015.11.131
- Borchard M.A., 1996. Nutrients. Pages 98-120 in R. J. Stevenson, M.L. Bothwell & R.L. Lowe, editors. Algal ecology. Academic Press, Inc., San Diego, California.
- Bornhauser K., 1913. Die Tierwelt der Quellen in der Umgebung Basels [The fauna of the springs in the vicinity of Basel, Switzerland]. Int Revue ges Hydrobiol Hydrogr Suppl 5(3):1–90
- Botosaneanu L., 1998. Studies in crenobiology; the biology of springs and springbrooks. Backhuys Publishers, Leiden
- Bourg, A.C.M., 1988, Metal in aquatic and terrestrial systems: Sorption, speciation, and mobilization, in Salomons, W., and Forstner, U., eds., Chemistry and biology of solid waste: Berlin, Springer-Verlag, p. 3-32. Brown, S.L., Chaney, R.L., Angle, J.S., and Baker, A.J.M., 1995, Zinc and cadmium uptake by hyperaccumulator
- Bradl H., 2002. Heavy Metals in the Environment: Origin, Interaction and Remediation Volume 6. London: Academic Press.
- Bryan G.W., Langston W.J., Hummerstone L.G. and Burt G.R., 1985. A guide to the assessment of heavy metal contamination in estuaries using biological indicators. Mar Biolo Assoc UK; 4: 91–110.
- Burton G.A., 2002. Sediment quality criteria in use around the world. Institute for Environmental Quality, Wright State University, Dayton, Ohio 45435, USA. Limnology (2002) 3:65–75
- Campbell P.G.C., Chapman P.M., Hale B.A., 2006. Risk assessment of metals in the environment. Issues in Environment Science and Technology, 22: 102:131.
- Cantonati M., Füreder L., Gerecke R., Jüttner I., Cox E.J., 2012. Crenic habitats, hotspots for freshwater biodiversity conservation: toward an understanding of their ecology. Freshw. Sci. 31:463-480.

- Cantonati M., Gerecke R., Bertuzzi E., 2006. Springs of the Alps – sensitive ecosystems to environmental change: from biodiversity assessments to long-term studies. Hydrobiologia, 562, 59-96.
- Carvalho A., Schropp S., 2002. Development of an Interpretive Tool for Assessment of Metal Enrichment in Florida Freshwater Sediment. Florida Department of Environmental Protection.
- Cattaneo, A., Kerimian, T., Roberge, M. Marty, J. (1997). Periphyton distribution and abundance on substrata of different size along a gradient of stream trophy. Hydrobiologia 354:101-110.
- Champion K.M., Starks R., 2001. The Hydrology and Water Quality of Select Springs in the Southwest Florida Water Management District
- Chaney R.L., 1988, Metal speciation and interaction among elements affect trace element transfer in agricultural and environmental food-chains, in Kramer, J.R. and Allen, H.E., eds., Metal Speciation: Theory, Analysis, and Application, Lewis Publications, Boca Raton, Fla., p. 219-259.
- Chapman D., Kimstach V., in UNESCO, WHO, UNEP, 1996. Water Quality Assessments - A Guide to Use of Biota, Sediments and Water in Environmental Monitoring - Second Edition. ISBN 0 419 21590 5 (HB) 0 419 21600 6 (PB). Chapter 3-Selection of water quality variables. World Wide Web URL: http://www.who.int/water_sanitation_health/resourcesquality/wqachapter3.pdf
- Chapman P.M., Wang F., Janssen C., Persoone G., Allen H.E., 1998. Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability, risk assessment, and remediation. Can J Fish Aquat Sci; 55(10): 2221–2243. doi:10.1139/f98-145
- Clarke F.W., Washington H.S., 1924. The composition of the Earth's crust. U.S. Geol. Survey, Profess. Paper 127.
- Clarkson T.W., Fishbein L., Geldmacher-von Mallinckrodt M., Piscator M., Schlipköter H.W., Stoeppler M., Stumm W., Sunderman Jr. F.W., 1991. Metals and Their Compounds in the Environment. Occurence, Analysis, and Biological relevance. Edited by Merian E. VCH Verlagsgesellschaft mbH, Weinheim.
- Clements WH, Carlisle DM, Lazorchak JM, Johnon PH, 2000. Heavy metals structure benthic communities in Colorado mountain streams. Ecol Appl; 10 (2): 626-38.
- Crawley M. J., 2013. The R book, Second edition (Ed.) Wiley, Chichster, West Sussex, United Kingdom.
- Dahiya S., Kaur A., 1999. Physico chemical characteristics of underground water in rural areas of Tosham subdivisions, Bhiwani district.
- Danks H.V., Williams D.D., 1991. Arthropods of springs, with particular reference to Canada: Synthesis and needs for research. In Williams, D. D. & H. V. Danks (eds), Arthropods of springs, with particular reference to Canada. Memoirs of the Entomological Society of Canada 155: 203–217.
- Dao Ligang, Morrison Liam , Kiely Ger, Zhang Chaosheng, 2012. Spatial distribution of potentially bioavailable metals in surface soils of a contaminated sports ground in Galway, Ireland. Environ Geochem Health DOI 10.1007/s10653-012-9478-7
- Davutluoglu, O.I., Seckin, G., Ersu, C.B., Yilmaz, T., Sari, B., 2011. Heavy metal content and distribution in surface sediments of the Seyhan River, Turkey. Journal of Environmental Management 92: 2250- 2259. doi: http://dx.doi.org/10.1016/j.jenvman.2011.04.013.
- De Lange Peter J., Norton David A., Lord Janice M., Given David R., 1994. Over-collecting: an overlooked factor in the decline of plant taxa. Publisher: International Bureau for Plant Taxonomy and Nomenclature. Pag. 181-185. World Wide Web URL: http://www.water.ncsu.edu/watershedss/info/macroinv.html
- De Martinez G.M.M, Barcarolli I.F., De Menezes E.J., Giacimin M.M., Wood C.M., Bianchini A., 2011. Acute toxicity, accumulation and tissue distribution of copper in the blue crab Callinectessapidus acclimated to different salinities: In vivo and in vitro studies. Aquatic toxicology 101: 88-99.
- Díaz de Alba M., Galindo-Riano M.D., Casanueva-Marenco M.J., García-Vargas M., C.M., 2011. Kosore Assessment of the metal pollution, potential toxicity and speciation of sediment from Algeciras Bay (South of Spain) using chemometric tools J. Hazard. Mater., 190, pp. 177–187.
- Dirilgen, 2000. Accumulation of Heavy Metals in Freshwater Organisms: Assessment of Toxic Interactions, 173-179.

- Döring M., 2002. Ecological Assessment of Springs and Spring Brooks in the Swiss National Park: Combining Fieldwork with Geodesy (GPS/Tachymetry) and GIS. Swiss Federal Institute for Environmental Science and Technology (EAWAG), Germany.(http://edoc.unibas.ch/911/1/DissB_8611.pdf)
- Dumnicka E. and Rozkowski J., 2010. Natural and antrophogenic factors that participate in the forming of the spring and benthic invertebrates in the Karst area of Cracow-Czestochowa upland (Poland). Biuletyn Panstwowego Instytutu Geologicznego, 441: 139-144.
- Dumnicka E., Galas J., Koperski P., 2007. Benthic invertebrates in karst springs: does substratum or location define communities?. Int. Rev. Hydrobiology, 92, 4/5: 452-464.
- Eggleton J., Thomas K.V., 2004. A review of factors affecting the release and bioability to contaminants during sediment disturbance events. Enironmental International, 30: 937-980
- Erman N.A., 2002. Lessons from a Long-term Study of Springs and Spring Invertebrates (Sierra Nevada, California, U.S.A.) and Implications for Conservation and Management. Department of Wildlife, Fish, and Conservation Biology. University of California, Davis. World Wide Web URL: https://www.dri.edu/images/stories/conferences_and_workshops/spring-fed-wetlands/spring-fed-wetlands-erman.pdf
- Erman N.A., Erman D.C., 1995. Spring permanence, Trichoptera species richness and the role of drought. Journal of the Kansas Entomological Society, 68, 50–64.
- Fatoki O.S., 1996. Trace zinc and copper concentration in roadside surface soils and vegetation–measurement of local atmospheric pollution in Alice, South Africa. Environment International, 22(6), 759–762
- Ferrington L.C., 1995. Biodiversity of aquatic insects and other invertebrates in springs: introduction. J Kans Entomol Soc 68:1–3
- Ferrington L.C., 1998. Generic composition of chironomid fauna in springs of North America. In: Botosaneanu L. Studies in Crenobiology. Backhuys Publishers, Leiden. pp 140-155.
- Filgueiras A.V., Lavilla I., Bendicho C., 2002. Chemical sequencing extraction for metal partitioning in environmental solid samples J. Environ. Monitor., 4, pp. 823–857
- Finžgar N., Tlustoš P., Leštan D., 2007. Relationship of soil properties to fractionation, bioavailability and mobility of lead and zinc in soil. Project No. BI-CZ/06-07-007. Plant Soil Environ., 53, 2007 (5): 225–238
- Fischer J., Fischer F., Schnabel S. & Bohle H.W., 1998. Spring fauna of the Hessian Mittelgebirge: Population structure, adaptative strategies, and relations to habitats of the macroinvertebrates, as exemplified by springs in the Rhenisch metamorphic shield and in the East-Hessian sandstone plate. In: Studies in Crenobiology. The biology of springs and springbrooks (Ed L. Botosaneanu), pp. 182-199. Backhuys Publishers, Leiden.
- Flemming, C., Trevors, J., 1989. Copper toxicity and chemistry in the environment: a review. Water, Air, Soil Pollut. 44, 143e158.
- Ford D.C., Williams P.F., 2007. Karst geomorphology and hydrology. Wiley, Hoboken, NJ
- Forstner U., Prosi F., Ravera O., 1979. Heavy Metal Pollution in Freshwater Ecosystems, Biological Aspects of Freshwater Pollution. Pergamon, Amsterdam pp. 129-161.
- Freeze R. A., Cherry J. A., 1979. Groundwater. Prentice Hall, Inc., Englewood Cliffs, New Jersey.
- Fu J., Zhao C., Luo Y., Liu C., Kyzas G.Z., Luo Y., Zhao D., An S., Zhu H., 2014. Heavy metals in surface sediments of the Jialu River, China: their relations to environmental factors. Hazard Mater 2014; 270: 102–109. doi:10.1016/j.jhazmat..01.044
- Fu Zhiyou, Wu Fengchang, Chen Lulu, Xu Bingbing, Feng Chenglian, Bai Yingchen, Liao Haiqing, Sun Siyang, Giesy John, Guo Wenjing, 2016. Copper and zinc, but not other priority toxic metals, pose risks to native aquatic species in a large urban lake in Eastern China. Environmental Pollution 1069-1076. Doi: http://dx.doi.org/10.1016/j.envpol.2016.09.007
- Fu, G., Allen, H.E., Cao, Y., 1992. The importance of humic acids toproton and cadmium binding in sediments. Environ. Toxicol. Chem. 11, 1363–1372.

- Gambrell R.P., Wiesepape J.B., Patrick W.H., Jr., Duff M.C., 1991, The effects of pH, redox, and salinity on metal release from a contaminated sediment: Water, Air, and Soil Pollution, v. 57-58, p. 359-367.
- George C.M., Goyer, R. A., 1978. Metallothioneins and their role in the metabolism and toxicity of metals. Life Sciences, 23, 1–9
- Gerecke R., 1991. Taxonomische, faunistische und ökologische Untersuchungen an Wassermilben aus Sizilien unter Berücksichtigung anderer aquatischer Invertebraten. Lauterbornia, 7, 1–304.
- Gerecke R., Franz H., Cantonati M., 2009. Invertebrate diversity in springs of the National Park Berchtesgaden (Germany): relevance for long-term monitoring. Verh. Internat. Verein. Limnol. 30:1229-1233.
- Gheorghe S., Stoica C., Vasile G.G., Nita-Lazar M., Stanescu E. and Lucaciu I., 2017. Metals Toxic Effects in Aquatic Ecosystems: Modulators of Water Quality. http://dx.doi.org/10.5772/65744
- Gillis, T.E., 1997. Certificate of Analysis Standard Reference Material 2710 Montana Soil (highly elevated trace element concentrations). National Institute of Standards and Technology, Gaithersburg, MD. 5 pp.
- Glazier D.S., 1991. The fauna of North American temperate cold springs: patterns and hypotheses. Freshwater Biology, 26, 527–542.
- Glazier D.S., Gooch J.L., 1987. Macroinvertebrate assemblages in Pennsylvania (U.S.A.) springs. Hydrobiologia 150:33–43
- Graening G.O., Brown A.V., 2000. Trophic dynamics and pollution effects in cave springs cave, Arkansas. Arkansas Water Resources center publication. Msc-285. URL: http://arkansas-water-center.uark.edu/publications/msc/MSC285.PDF
- Grasby S.E., Londry K.L., 2007. Biogeochemistry of hypersaline springs supporting a mid-continent marine ecosystem: an analogue for Martian Springs. Astrobiology 7:662–683
- Griffiths R.E., Springer A.E., Anderson D.E., 2008. The morphology and hydrology of small spring-dominated channels. Geomorphology (in press)
- Habdija I., Radanovic I., Primc-Habdija B. & Špoljar M., 2002. Vegetation cover and substrate type as factors influencing the spatial distribution of Trichopterans along a karstic
- Hahn H.J., 2000. Studies on classifying of undisturbed springs in southwestern Germany by macrobenthic communities. Limnologica 30:247–259
- Hill W.A. 1996. Effects of light. Pages 121-150 in R. J. Stevenson, M.L. Bothwell & R.L. Lowe, editors. Algal ecology. Academic Press, Inc., San Diego, California.
- Hnatukova P., 2007. Distribution of heavy metals in the environment of small urban streams. Ph.D. thesis, Charles University, Prague, Czech Republic.
- Hoffsten P.O., Malmqvist B., 2000. The macroinvertebrate fauna and hydrogeology of springs in central Sweden. Hydrobiologia, 436, 91–104.
- Horowitz, A.J. 1991. A Primer On Sediment-Trace Element Chemistry. 2nd Ed. Lewis Publishers, Inc. Michigan. 136 p. http://dx.doi.org/10.1016/j.microc.2009.12.015.
- Hynes H.B.N., 1970. The ecology of running waters. University of Toronto Press, Toronto
- Idris A.M., Eltayeb M.A.H., Potgieter-Vermaak S.S., Grieken R., Potgieter J.H., 2007. Assessment of heavy metal pollution in Sudanese harbours along the Red Sea coast. Microchemical. J 87: 104-112.
- Illies J., 1952. Die Mölle. Faunistisch-ökologische Untersuchungen an einem Forellenbach im Lipper Bergland. Archiv für Hydrobiologie, 46, 424–612.
- Ilmonen J., Paasivirta L., 2005. Benthic macrocrustacean and insect assemblages in relation to spring habitat characteristics: patterns in abundance and diversity. Hydrobiologia 533:99–113
- Ingersoll CG, Haverland PS, Brunson EL, Canfield TJ, Dwyer FJ, Henke CE, Kemble NE, Mount DR, Fox RG, 1996. Calculation and evaluation of sediment effect concentrations for the amphipod, Hyalella azteca and the midge, Chironomus riparius. J Great Lakes Res 22:602–623
- Inyang Hilary I., Daniels John L., 2009. ENVIRONMENTAL MONITORING – Vol. II - Deposition of Pollutants and Their Impacts on Fisheries - Jana B.B. Encyclopedia of Life Support Systems (EOLSS).

- Iordache V., 2009. Ecotoxicology of heavy metals in Danube meadow. Bucharest, Romania; Ars Docenti. ISBN 978-973-558-233-3.
- Jain C.K., 2002. A hydro-chemical study of a mountainous watershed: the Ganga, India. Water Res. 3. p. 1262-1274.
- Jain C.K., 2004. Metal fractionation study on bed sediments of River Yamuna, India. Water Res., 38, pp. 569–578.
- Janetzky W., 1994. Distribution of the genus Gammarus (Amphipoda: Gammaridae) in the River Hunte and its tributaries (Lower Saxony, northern Germany). Hydrobiologia 294: 23-34.
- Kabala C, Singh BR, 2001. Fractionation and mobility of cooper, lead and zinc in soil profiles in the vicinity of a copper smelter. J Environ Qual; 30: 485-92.
- Karan V., Vitorovic S., Tutundzic V., Poleksic V., 1998. Functional Enzymes Activity and Gill Histology of Carp after Copper Sulfate Exposure and Recovery. Ecotoxicology and Environmental Safety 40: 49-55.
- Kataria H. C., Quershi H. A., Iqbal S. A., Shandilya A. K., 1996. Assessment of water quality of Kolar reservoir in Bhopal (M.P.). Pollution Research. 15(2), p. 191-193.
- Kierczak J., Neel C., Aleksander-Kwaterczak U., Helios-Rybicka E., Bril, H., Puziewicz J., 2008. Solid speciation and mobility of potentially toxic elements from natural and contaminated soils: A combined approach. Chemosphere 73: 776-784. doi: http://dx.doi.org/10.1016/j.chemosphere.2008.06.015.
- Klaassen, C.D., J. Liu and S. Choudhuri, 1999. Metallothionein: An intracellular protein to protect against cadmium toxicity. Annu. Rev. Pharmacol. Toxicol., 39: 267-274.
- Köhler H-R, Sandu C., Scheil V., Nagy-Petrica E.M., Segner H., Telcean I., Stan G., Triebskorn R., 2007. Monitoring pollution in river Mureș, Romania, Part III: biochemical effect markers in fish and integrative reflection. Environ Monit Assess; 127(1): 47–54. doi:10.1007/s10661-006-9257-y
- Kominkova D., 2001. Pollution of Aquatic Ecosystems by Heavy Metals – the Kocaba and the Tocnicky Stream. PhD. thesis. Charles University, Prague, Czech Republic.
- Komínková, D., Nábělková, J., 2007. Effect of urban drainage on bioavailability of heavy metals in recipient. Water Science & Technology. 2007, vol. 56, no. 9, p. 43-50. (Published in UK)
- Krishnamurthy R., 1990. Hydro-biological studies of Wohar reservoir Aurangabad (Maharashtra State) India, Journal of Environmental Biology, 11(3), 335-343
- Lane T.W., Morel F.M.M., 2000. A biological function for cadmium in marine diatoms. Proc. Natl. Acad. Sci. USA, 97: 4627-4631.
- Lee G. F. and Jones-Lee, A. 1993. Sediment Quality Criteria: Numeric Chemical- vs. Biological Effects-Based Approaches, Proceedings of the Water Environment Federation National Conference, Anaheim, pp. 389-400.
- Lindegaard C., Brodersen K.P., Wiberg-Larsen P. & Skriver J., 1998. Multivariate analyses of macrofaunal communities in Danish springs and springbrooks. In: Studies in Chapter 5: Where a springhead becomes a springbrook - a regional zonation of springs 79 crenobiology. The biology of springs and springbrooks. (Ed L. Botosaneanu), pp. 201-220. Backhuys Publishers, Leiden.
- Long E.R., MacDonald D.D., 1998. Recommended uses of empiricallyderived sediment quality guidelines for marine and estuarine ecosystems. Hum Ecol Risk Assess 4:1019–1039
- Long E.R., MacDonald D.D., Sever C.G., Hong C.B., 2000 Classifying probabilities of acute toxicity in marine sediments with empirically derived sediment quality guidelines. Environ Toxicol Chem 19:2598–2601
- Long E.R., Morgan L.G., 1991. The potential for biological effects of sediment-sorbed contaminants tested in the National Status and Trends Program. National Oceanic and Atmospheric Administration Technical Memorandum, NOS OMA 52, Seattle, WA, USA
- Luoma S.N., 1989. Can we determine the biological availability of sediment-bound trace elements?: Hydrobiologia, v. 176/177, p. 379-396.
- MacDonald D.D., Ingersoll C.G., Berger T.A., 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch Environ Contam Toxicol 39:20–31

- Malard F., Tockner K., WardbJ. V., 1999. Shifting dominance of subcatchment water ources and flow paths in a glacial floodplain, Val Roseg, Switzerland. Arctic, Antarctic, and Alpine Research 31:135-150.
- Malek S., Krakowian K., 2012. The effect of deforestation on spring water chemistry on Skrzyczne (Silesian Beskid Mountains, Poland). Department of Forest Ecology, Faculty of Forestry, University of Agriculture in Krakow, Poland. Journal of forest science, 58 (7): 308–313.
- Marsalek Jiri, Rochfort Quintin, Grapentine Lee, 2005. Aquatic habitat issues in urban stormwater management: challenges and potential solutions. National Water Research Institute, 867 Lakeshore Road, Burlington, Ontario, Canada L7R 4A6.
- Marsalek, J., Rochfort, Q., Mayer T., Servos, M., Dutka, B., Brownlee, B., 1999. Toxicity testing for controlling urban wet-weather pollution: advantages and limitations. Urban Water 1, 91-103.
- Marsden I., Rainbow P. S., 2004. Does the accumulation of trace metals in crustaceans affect their ecology. Journal of experimental Marine Biology and Ecology, 300: 373-408.
- Mattson R.A., Eppler J.H., Hein M.K., 1995. Description of benthic community in karst, spring-fed streams of north central Florida. Pages 18-41 in L. C. Ferrington, editor. Biodiversity of aquatic insects and other invertabrates in springs. Journal of the Kansas Entomological Society.
- McCabe D.J., 1998. Biological communities in springbrooks. In: Botosaneanu L (ed) Studies in Crenobiology: The biology of springs and springbrooks. Backhuys, Leiden, The Netherlands
- McCaffrey Stephanie, 2013. Namoi Catchment Management Authority-Water Quality Parameters and Indicator. New South Wales: Retrieved from: http://www.sswm.info/sites/default/files/reference_attachments/MCCAFFREY%20ny%20Water%20Quality%20Parameters%20&%20Indicators.pdf
- Meinzer O.E., 1923. Outline of ground-water hydrology, with definitions. US Geol Surv Water Suppl Pap 114 494
- Meybeck M., Kuusisto E., Mäkelä A. and Mälkki E., 1996. Water Quality Monitoring - A Practical Guide to the Design and Implementation of Freshwater. Quality Studies and Monitoring Programmes. Edited by Jamie Bartram and Richard Balance. Published on behalf of United Nations Environment Programme and the World Health Organization. doi:
- Meyer A., Meyer E.I., Meyer C., 2003. Lotic communities of two small temporary karstic stream systems (East Westphalia, Germany) along a longitudinal gradient of hydrological intermittency. Limnologica 33:271–279
- Milošković A., Branković S., Simić V., Kovačević S., Cirković M., Manojlović D., 2013. The accumulation and distribution of metals in water, sediment, aquatic macrophytes and fishes of the Gruža Reservoir, Serbia, Bull. Environ. Contam. Toxicol., Retrived from DOI 10.1007/ s00128-013-0969-8.
- Mohamed E.G., Hassan I.F., Mohamed H.A., Salem G.S., 2014. Metal pollution assessment in the surface sediment of Lake Nasser, Egypt. The Egyptian Journal of Aquatic Research. Vol (40), Issue 3, 2014, Pages 213–224. http://doi.org/10.1016/j.ejar.2014.09.004
- Mori N., Brancelj A., 2006. Macroinvertebrate communities of karst springs of two river catchments in the Southern Limestone Alps (the Julian Alps, NW Slovenia). Aquatic Ecology, 40, 69-83.
- Morillo J., Usero J., Gracia I., 2007. Potential mobility of metals in polluted coastal sediments in two Bays of southern Spain. J. Coast. Res., 23 (No. 2), pp. 352–361.
- Morrissette D.G., Mavinic D.S., 1978. BOD Test Variables. Journal of Environment: Engg. Division, EP, 6, 1213-1222.
- Moss B., 1972. Studies on Gull Lake, Michigan II. Eutrophication evidence and prognosis, Fresh Water Biology, 2, p. 309-320.
- Nábělková J., 2005. Heavy metals mobility in small urban creeks environment. Ph.D thesis CTU, Prague, Czech Rep.
- Nábělková J., Komínková D., 2012. Trace Metals in the Bed Sediment of Small Urban Streams. The Open Environmental & Biological Monitoring Journal, 2012, 5, (Suppl 1: M5) 48-55. CTU in Prague, Czech Republic.

- Naji A., Ismail A., Ismail A.R., 2010. Chemical speciation and contamination assessment of Zn and Cd by sequential extraction in surface sediment of Klang River, Malaysia. Microchem. J. 95: 285-292. doi:
- Nemati K, Abu Bakar N.K., Radzi Abas M, Sobhanzadeh E., 2011. Speciation of heavy metals by modified BCR sequential extraction procedure in different depths of sediments from Sungai Buloh, Selangor, Malaysia. Journal of Hazardous Materials, 192(1): 402-410.
- Nguyen H.L., Braun M., Szaloki I., Baeyens W., Van Grieken R., Leermakers M., 2009. Tracing the metal pollution history of the Tisza River through the analysis of a sediment depth profile Water Air Soil Pollut., 200, pp. 119–132.
- Odum H.T. 1971. Fundamentals of Ecology. Saunders, Philadelphia.
- Odum H.T., 1957. Trophic structure and productivity of Silver Springs, Florida. Ecological Monographs, 27, 55–112.
- Okoro H.K., Fatoki O.S., Adekola F.A., Ximba B.J., Snyman R.G., 2012. A Review of Sequential Extraction Procedures for Heavy Metals Speciation in Soil and Sediments. 1: 181. doi:10.4172/scientificreports.181
- Orendt C., 2000. The chironomid communities of woodland springs and spring brooks, severely endangered and impacted ecosystems in a lowland region of eastern Germany (Diptera: Chironomidae). Journal of Insect Conservation, 4, 79-91.
- Passos E.A., Alves J.C., Santos I.S., Alves J.P., Garcia C.A.B., Costa A.C.S., 2010. Assessment of trace metals contamination in estuarine sediments using a sequential extraction technique and principal component analysis Microchem. pp. 50–57.
- Patil P.N., Sawant D.V., Deshmukh R.N., 2012. Physico-chemical parameters for testing of water – A review. International Journal of Environmental Sciences. Vol 3, (3). ISSN 0976 – 4402
- Patil, V.T., Patil, R.R. 2010. Physicochemical analysis of selected groundwater samples of Amalner Town in Jalgaon District, Maharashtra, India. E J. Chem., Vol. 7(1), 111-116.
- Pekey H.P., Karakas S., Ayberk L., Tolun L., Bakoglu M., 2006. Ecological Risk Assessment Using Trace Elements from Surface Sediments of Izmit Bay (Northeastern Marmara Sea) Turkey. Marine Pollution Bulletin, 48, 9-10, 946-953.
- Perez-Lopez R., Nieto J.M., Lopez-Coto I., Aguado J.L., Bolivar J.P., Santisteban M., 2010. Dynamics of contaminants in phosphogypsum of the fertilizer industry of Huelva (SW Spain): From phosphate rock ore to the environment. Applied Geochemistry 25: 705-715. doi: http://dx.doi.org/10.1016/j.apgeochem.2010. 02.003.
- Perla B.S., Stevens L.E.. 2008. Biodiversity and productivity at an undisturbed spring in comparison with adjacent grazed riparian and upland habitats. In: Stevens LE, Meretsky VJ (eds) Aridland springs in North America: ecology and conservation. University of Arizona Press, Tucson
- Peterson C.G., 1996. Response of bentic algal communities to natural physical disturbance. Pages 375-398 in R. J. Stevenson, M.L. Bothwell & R.L. Lowe, editors. Algal Ecology. Academic Press, Inc., San Diego, California.
- Pickett S.T.A., White P.S. (Eds) (1985) The ecology of natural disturbance and patch dynamics. Academic Press, San Diego.
- Pöckl M., Webb B.W., Sutcliffe D.W., 2003. Life history and reproductive capacity of Gammarus fossarum and G. roeseli (Crustacea: Amphipoda) under naturally fluctuating water temperatures: a simulation study. Freshwater Biology 48: 53-66.
- Prasad M., Muralidhara Reddy B., Ramakrishna Reddy M., Sunitha V., 2014. Studies on physicochemical parameters to assess the water quality in Obulavaripalli Mandal of YSR (Kadapa) District, Andhra Pradesh, India. ISSN: 2347-3215 Volume 2 Number 12, pp. 31-41.
- Prato E., Parlapiano I., Biandolino F., 2013. Sublethal effects of copper on some biological traits of the amphipod Gammarus aequicauda reared under laboratory conditions. Chemosphere, 93:6: 1015-1022.
- Premlata V., 2009. Multivariant analysis of drinking water quality parameters of lake Pichhola in Udaipur, India. Biological Forum, Biological Forum- An International Journal, 1(2), pp 97-102.
- Prokop Z., Vangheluwe M.L., Van Sprang P.A., Janssen C.R., Holoubeka I., 2002. Mobility and toxicity of metals in sandy sediments deposited on land. Ecotoxicology and Environmental Safety 54 (2003) 65–73

- Prosi Fritz, 1989, Factors controlling biological availability and toxic effects of lead in aquatic organisms: The Science of the Total Environment, v. 79, p. 157-169.
- Prusty, B.G., Sahu, K.C., and Godgul, G., 1994, Metal contamination due to mining and milling activities at the Zawar zinc mine, Rajasthan, India. 1. Contamination of stream sediments: Chemical Geology, v. 112, p. 275-292.
- Pueyo, M., Mateu, J., Rigol, A., Vidal, M., Lo´pez-Sa´nchez, J.F., Rauret, G. (2008) Use of the modified BCR three-step sequential extraction procedure for the study of trace element dynamics in contaminated soils. Environmental Pollution 152: 330-341. doi: http://dx.doi.org/10.1016/j.envpol.2007.06.020.
- Pueyo, M., Rauret, G., Lück, D., Yli-Halla, M., Muntau, H., Quevauviller, Ph., López-Sánchez, J.F., 2001. Certification of the extractable contents of Cd, Cr, Cu, Ni, Pb and Zn in a freshwater sediment following a collaboratively tested and optimized three-step sequential extraction procedure. J. Environ. Monit. 3, 243–250.
- Quevauviller P, Olazabal J., 2002. Links between the water framework directive,
- Rand G.M., 1995. Fundamentals of Aquatic Toxicology: Effects, Environmental Fate and Risk Assessment. 2nd ed. North Palm Brach: Taylors & Francis.
- Rankama Kalervo, Sahama Th. G., 1968. Geochemistry. Sixth impression. Printed in the United State of America. The University of Chicago Press, Ltd., London W. C. 1. Pag. 264-281
- Rao C.R.M., Sahuquillo A., Lopez-Sanchez J.F., 2010. Comparison of single and sequential extraction procedures for the study of rare earth elements remobilisation in different types of soils. Analytica Chimica Acta 662(2): 128-136. doi: http://dx.doi.org/10.1016/j.aca.2010.01.006.
- Ratuzny T., Gong Z., Wilke B.M., 2009. Total concentrations and speciation of heavy metals in soils of the Shenyang Zhangshi Irrigation Area, China Environ. Monitor. Assess., 156, pp. 171–180
- Rauret G., Lopez-Sanchez J.F., Sahuquillo A., Rubio R., Davidson C., Ure A., Quevauviller P., 1999. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. Journal of Environmental Monitoring 1: 57-61. doi: http://dx.doi.org/10.1039/a807854h.
- Rauret Gemma, 1998. Extraction procedures for the determination of heavy metals in contaminated soil and sediment. Elsevier Science B.V. PII S0039-9140(97)00406-2 Talanta 46 (1998) 449–455.
- Raži´c S., Dogo S., 2010. Determination of chromium in Mentha piperita L. and soil by graphite furnace atomic absorption spectrometry after sequential extraction and microwave-assisted acid digestion to assess potential bioavailability. Chemosphere 78(4): 451-456. doi: http://dx.doi.org/10.1016/j.chemosphere.2009.10.028.
- Resh V.H., 1983. Spatial differences in the distribution of bentic macroinvertebrates along a springbrook. Aquatic Insects 5:193-200.
- Rieuwerts J.S., Thornton I., Farago M.E., Ashmore M.R., 1998. Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals. Chem. Spec. Bioavail., 10: 61–75.
- Rinaldi F., Komínková D., Berchová K., Daguenet J., Pecharová E., 2017. Stable cesium(^{133}Cs) uptake by *Calla palustris* from different substrates. Ecotoxicology and Environmental safety 139 (2017) 301-307. CULS in Prague, Czech Republic.
- Roca J,R., Castillo M., 1993. Typology of the Pyrenean springs based on their biological communities. In: Actas VI Congreso Espan˜ol de Limnologı´a. Granada, Spain, pp 283–289
- Roca J.R., 1990. Tipologia fisico-quimica de las Fuentes de los Pireneos centrales: síntesis regional. Limnetica, 6, 57-78.
- Roesijadi G., Robinson W.E., 1994. Metal regulation in aquatic animals: mechanisms of uptake, accumulation, and release. In: Malins DC and Ostrander GK (eds). Aquatic toxicology: molecular, biochemical, and cellular perspectives. Lewis Publishers: Boca Raton. P. 387–420. ISBN-13: 978-0873715454. ISBN-10: 0873715454.
- Rolland T., Fayolle S., Cazaubon A., Pagnetti S., 1997. Methodical approach to distribution of epiliptc and drifting algae communities in a French subalpine river: Inferences on water quality assessment. Aquatic Science 59:57-73.
- Roosa S., Prygiel E., Lesven L., Wattiez R., Gillan D., Ferrari BJ., Criquet J., Billon G., 2016. On the bioavailability of trace metals in surface sediments: a combined

geochemical and biological approach. Environ Sci Pollution Res; 23(11): 10679–10692. doi:10.1007/s11356-016-6198-z

- Rosado D., Usero J., Morillo J., 2016. Assessment of heavy metals bioavailability and toxicity toward Vibrio fischeri in sediment of the Huelva estuary. Chemosphere; 153: 10–17. doi:10.1016/j.chemosphere.2016.03.040
- Rosenberg D.M., Resh V.H., 1993. Freshwater Biomonitoring and Benthic Macroinvertebrates, Chappman & Hall, New York, NY, USA.
- Ruqia Nazir, Muslim Khan, Muhammad Masab, Hameed Ur Rehman, Naveed Ur Rauf, Surrya Shahab, Nosheen Ameer, Muhammad Sajed, Mohib Ullah, Muhammad Rafeeq, Zeenat Shaheen, 2015. Accumulation of Heavy Metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-chemical parameters of soil and water Collected from Tanda Dam kohat. Sci. and Res. Vol. 7(3), 89-97.
- Ruyters S., Salaets P., Oorts K., Smolders E., 2013. Copper toxicity in soils under established vineyards in Europe: A survey. Science of the Total Environment 443: 470-477.
- Ryan M., Meiman J., 1996. An examination of short term variations in water quality at a karst spring in Kentucky. Groundwater 34 (1):23-30.
- Rychla Anna, Buczyńska Edyta, Szczucińska Anna M., 2015. The Environmental Requirements of Crunoecia Irrorata (Curtis, 1834) (Trichoptera: Lepidostomatidae) and the Potential of the Species for Use as an Indicator: An Example from the Vistulian Glaciation Area. Journal of Limnology, Vol 74, No 3. DOI: 10.4081/jlimnol.2015.1108
- Sada D.W., Vinyard GL., 2002. Anthropogenic changes in historical biogeography of Great Basin aquatic biota. In: Great Basin aquatic systems history. Smithsonian Contributions to Earth Science 33, Smithsonian Institute, Washington, DC, pp 227–293
- Sahuquillo A., Rigol A., Rauret G., 2003. Overview of the use of leaching, extraction tests for risk assessment of trace metals in contaminated soils and sediments. Trend Analytical Chemistry 22: 152-159.
- Sakan, S., Grzetic´ , I., DorCevic´ , D., 2007. Distribution and fractionation of heavy metals in the Tisa (Tisza) River Sediments. Environmental Science and Pollution Research International 14 (4), 229–236.
- Salem, Z.B., Ayadi, H., 2016. Heavy metal accumulation in Diplodus Annularis, Liza aurata, and Solea vulgaris relevant to their concentration in water and sediment from the southwestern Mediterranean (coast of Sfax). Environ. Sci. Pollut. Res. http://dx.doi.org/10.1007/s11356-016-6531-6.
- Salomons, W., 1995, Environmental impact of metals derived from mining activities: Processes, predictions, prevention: Journal of Geochemical Exploration, v. 52, p. 5-23.
- Sambugar B., Dessi G., Sapelza A., Scenico A., Thaler B. & Veneri A. (2006) Südtiroler Quellfauna. Autonome Provinz Bozen.
- Saracoglu S., Soylak M., Elci L., 2009. Extractable trace metals contents of dusts of air filters from vehicles by sequential extraction procedure. Journal of the Association of Official Agricultural Chemists 92: 1196-1202.
- Sazzad, H.M., Wijaya, A.R., Tanaka, K., Ohde, S., 2010. Environmental effects on the stable carbon and oxygen isotopic compositions and skeletal density banding pattern of Porites coral from Khang Khao Island, Thailand. Afric. J. Biotechnol. 9 (33), 5373–5382.
- Scarsbrook M.R., Barquín J., Gray D., 2007. New Zealan coldwater springs and their biodiversity. In: Department of Conservation. Science for Conservation 278: Wellington, New Zealand, pp 74
- Schindler H., 2004. Bewertung der Auswirkungen von Umweltfaktoren auf die Struktur und Lebensgemeinschaften von Quellen in Rheinland-Pfalz. Fachgebiet Wasserbau und Wasserwirtschaft, Universität Kaiserslautern, Bericht 17.
- Schmidlin Lara, 2015. Gammarus fossarum Exposed to Copper and Rising Temperatures. Spring Gammarids in Difficulties?. Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät
- Seen A.J., Larner B.L., Palmer A.S., Townsend A.T., 2008. A comparison of an optimised sequential extraction procedure and dilute acid leaching of elements in anoxic sediments, including the effects of oxidation on sediment metal partitioning. Anal. Chim. Acta, 608, pp. 147–157.

- Shrinivasa R.B., Venkateswaralu P., 2000. Physicochemical analysis of selected groundwater samples, Indian J. Environ. Prot. 20. p. 161-164.
- Shyamala R., Shanthi M., Lalitha P., 2008. Physicochemical analysis of borewell water samples of Telungupalayam area in Coimbatore District, Tamilnadu, India, E-J.Chem., 5. p. 924-929
- Simpson S., Batley G., Chariton A., Stuber J., King C., Chapman J., Hyne R., Gale S., Roach A., Maher W., 2005. Handbook for sediment quality assessment, Environmental Trust, Canberra, ACT, Australia.
- Simpson S.L., King C.K., 2005. Exposure-Pathway Models Explain Causality in whole sediment toxicity tests. Environmental Science and Technology, 39: 837-843.
- Singh K.P., Mohan D., Singh V.K., Malik A., 2005. Studies on distribution and fractionation of heavy metals in Gomati river sediments – a tributary of the Ganges, India J. Hydrol., 312, pp. 14–27.
- Smith H., Wood P.J. & Gunn J. (2003) The influence of habitat structure and flow permanence on invertebrate communities in karst spring systems. Hydrobiologia, 510, 53-66.
- Smith K., Lavis, M.E., 1975. Enviromental influences on the temperature of a small upland stream. Oikos 26:228-236.
- Smith M.E., 1985. Populations and reproductive dynamics of Nais communis (Oligochaeta: Naididae) from a Wisconsin limnocrene. The American Midland Naturalist 114:152-157.
- Snoejink V.L., Jenkins D., 1980. Water chemistry. John Wiley & Sons, New York.
- Sorensen E.M.B., 1991. Metal Poisoning in Fish. CRC Press, Boston, MA., USA.
- Spence B.C., Lomnicky G.A., Hughes R.M., Novitzki R.P., 1996. An ecosystem approach to salmonid conservation. TR-4501-96-6057. ManTech Environmental Res. Services Corp., Corvallis, OR (available from the National Marine Fisheries Service, Portland, OR).
- Springer, A.E. and L.E. Stevens, 2009. Spheres of Discharge of Springs, Hydrogeology Journal, 17, 1: 83-94. doi: 10.1007/s10040-008-0341-y. [11, 23]
- Springer, A.E., L.E. Stevens, D.E. Anderson, R.A. Parnell, D.K. Kreamer, S.P. Flora, 2008. A comprehensive springs classification system: Integrating geomorphic, hydrogeochemical, and ecological criteria. in Aridland Springs in North America: Ecology and Conservation, Univ. Arizona Press, Tucson, eds. Stevens, L.E. and V.J. Meretsky. [-, 10]
- Steinmann P., 1915. Praktikum der Süsswasserbiologie. Teil 1: Die Organismen des fliessenden Wassers.
- Stevens L., 2007. Water and biodiversity on the Colorado Plateau. Plateau J 4:48–55
- Stumm W., Morgan J.J., 1996. Aquatic Chemistry, New York.
- Taylor, B., Kovats, Z., 1995. Review of artificial substrates for benthos sample collection, for Canada centre for mineral and energy technology. Available from: http://www.nrcan.gc.ca/mms/canmet-mtb/mmsl-lmsm/enviro/reports/3_2_1.pdf.
- Tchounwou Paul B., Yedjou Clement G., Patlolla Anita K., Sutton Dwayne J, 2014. Heavy Metals Toxicity and the Environment. Published in final edited form as: EXS. 2012; 101: 133–164. doi: 10.1007/978-3-7643-8340-4_6
- Tessier A., Campbell P.G.C., Bisson M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. Journal of Analytical Chemistry 51(7): 844- 851. doi: http://dx.doi.org/10.1021/ac50043a017.
- Toth J., 1966. Mapping and interpretation of field phenomena for groundwater reconnaissance in a prairie environment, Alberta, Canada. Bull Int Assoc Sci Hydrol 9:20–68
- Triebskorn R., Telcean I., Casper H., Farkas A., Sandu C., Stan G., Colărescu O., Dori T., Köhler H-R., 2008. Monitoring pollution in River Mureş, Romania, part II: metal accumulation and histopathology in fis. Environ Monit Assess; 141(1): 177–188. doi:10.1007/s10661-007-9886-9
- Tüzen M., 2003. Determination of trace metals in the River Yesilirmak sediments in Tokat, Turkey using sequential extraction procedure Microchem. J., 74, pp. 105–110.
- U.S. EPA – United States Environmental Protection Agency, 1996. The metal Translator: Guidance for Calculating a Total Recoverable Permit Limit from a Dissolved Criterion.

- U.S. EPA, 1999. US EPA 402-R-99-004A (Understanding Variation in PartitionCoefficient, Kd, Values. Volume I - Kd Model, Measurement Methods, and Application of Chemical Reaction Codes. Office of Air and Radiation, Washington DC: U.S.A.
- UNEP., 2010. Final review of scientific information on cadmium. Chemicals Branch, DTIE, United Nations Environment Programme.
- Upchurch, S. B., 1992. Quality of Waters in Florida's Aquifers. In G. L. Maddox, J. M. Lloyd, T. M. Scott, S. B. Upchurch, and R. Copeland (eds.), Florida Ground Water Quality Monitoring Program – Volume 2, Background Hydrogeochemistry, Florida Geological Survey, Special Publication No. 34. 364 p.
- Urbonas, B., Benik, B. 1995. Stream stability under a changing environment. In: Herricks, E.E. [Ed.] Stormwater runoff and receiving systems, CRC, Lewis Publishers, Boca Raton, ISBN 1-56670-159- 7, pp.77-101.
- Van der Kamp R.O., 1995. The hydrogeology of springs in relation to the biodiversity of spring fauna: a review. In: Biodiversity of aquatic insects and other invertebrates in sprins (Ed. L.C. Ferrington), pp. 4-17. Journal of the Kansas Entomological Society, 68.
- Vandas S.J., Winter T.C., Battaglin W.A., 2002. Water and the Environment. Published by the American Geological Institute in cooperation with Bureau of Reclamation, National Park Service, U.S. Army Corps of Engineers, USDA Forest Service, U.S. Geological Survey. pp. 42-44. doi: http://www.cns-eoc.colostate.edu/docs/water.pdf
- Verdonschot P.F.M., 1995. Typology of macrofaunal assemblages: a tool for the management of running waters in The Netherlands. Hydrobiologia, 297, 99-122.
- Von Fumetti S, Nagel P. 2011. A first approach to a faunistic crenon typology based on functional feeding groups. Journal of Limnology 70(Suppl. 1): 147-154.
- Von Fumetti S., 2008. Distribution, discharge and disturbance: new insights into faunal spring ecology. http://edoc.unibas.ch/911/1/DissB_8611.pdf
- Von Fumetti S., Nagel P., 2012. Discharge variability and its effect on faunistic assemblages in springs. Freshwater Science 31: 647-656.
- Wali Ahmed, Colinet Gilles, Ksibi Mohamed, 2014. Speciation of Heavy Metals by Modified BCR Sequential Extraction in Soils Contaminated by Phosphogypsum in Sfax, Tunisia. Engineering and Management 4(70): 14–26. doi:
- Wallace M.P., Alfaro C., 2001. Geologic/hydrogeologic setting and classification of springs. In: LaMoreaux PE, Tanner JT (eds) Springs and bottled waters of the world: ancient history, source, occurrence, quality and use. Springer, Berlin
- Wang W.X., Fisher N.S., 1997. Modeling metal bioavailability in marine mussels. Rev Environ Contam Toxicol; 151: 39–65. doi:10.1007/978-1-4612-1958-3_2
- Ward D.J., 2013. The response of marine benthic fauna exposed to continuous and pulsed exposures of contaminated sediment: a study of avoidance and toxicity. University of Wollongong
- Ward J.V., 1992. Aquatic Insect Ecology: 1. Biology and Habitat. John Wiley & Sons, New York.
- Ward J.V., 1994. Ecology of alpine streams. Freshwater Biology 32:277-294.
- Webb D.W., Wetzel M.J, Reed P.C., Philippe L.R. & Young, T.C., 1998. The macroninvertebrate biodiversity, water quality, and hydrogeology of ten karst springs in the Salem Plateau of Illinois. In: Studies in Crenobiology. The biology of springs and springbrooks (Ed L. Botosaneanu), pp. 39-48. Backhuys Publishers, Leiden. The Netherlands. 261 pp. ISBN 90-73348-04-8.
- Wetzel, M.J., and D.W. Webb. 2007. Springs of Illinois homepage. World Wide Web URL: http://www.inhs.uiuc.edu:80/~mjwetzel/SPOIL.hp.html (March 2017).
- WHO (World Health Organization), FAO, IAEA, 1996.. Trace Elements in Human Nutrition and Health. Switzerland: Geneva.
- Wijaya A.R., Ohde S., Shinjo R., Ganmanee M., Cohen M.D., 2016. Geochemical fractions and modeling adsorption of heavy metals into contaminated river sediments in Japan and Thailand determined by sequential leaching technique using ICP-MS. Arabian Journal of Chemistry (2016), http://dx.doi.org/10.1016/j.arabjc.
- Williams D.D., 1991. Life history traits of aquatic arthropods in springs. Memoirs of the entomological society of Canada 155:63-87.

- Williams D.D., Danks H.V., Smith I.M., Ring R.A., Cannings R.A.,1990. Freshwater springs: A national heritage. In: The entomological society of Canada, Supplement to the Bulletin, vol 22(1), pp 9
- Williams D.D., Williams N.E., Cao Y., 1997. Spatial differences in macroinvertebrate community structure in springs in southeastern Ontario in relation to their chemical and physical environments. Canadian Journal of Zoology, 75, 1404–1414
- Wojtkowska M., Bogacki J., Witeska A., 2016. Assessment of the hazard posed by metal forms in water and sediments. Sci Total Environ; 551–552: 387–392. doi:10.1016/j.scitotenv.2016.01.073
- Wright, D.A., Welbourn, P., 2002. Environmental Toxicology. Cambridge University Press.
- Yang, Z., Wang, Y., Shen, Z., Niu, J., Tang, Z., 2009. Distribution and speciation of heavy metals in sediments from the main stream, tributaries, and lakes of the Yangtze river catchment of Wuhan, China. Journal of Hazardous Materials 166: 1186-1194. doi: http://dx.doi.org/10.1016/j.jhazmat.2008.12.034.
- Yoder C., 1989. The development and use of biocriteria for Ohio surface waters. In: Flock, G.H. [Ed.] Proc. National Conf. Water Quality Standards for the 21st Century, U.S. EPA, Office of Water, Washington, D.C., pp. 139-146.
- Zalewski M., Wagner-£otkowska I., Robarts R.D., 2004. Integrated Watershed Management - Ecohydrology & Phytotechnology - Manual. International Hydrological Programme UNESCO (IHP), Regional Bureau for Science in Europe (UNESCO - ROSTE),http://www.unep.or.jp/ietc/publications/freshwater/watershed_manual/index.asp)
- Zemberyov´a M., Bartekova J., Hagarova I., 2006. The utilization of modified BCR three-step sequential extraction procedure for the fractionation of Cd, Cr, Cu, Ni, Pb and Zn in soil reference materials of different origins. Talanta 70: 973-978. doi: http://dx.doi.org/ 10.1016/j.talanta.2006.05.057.
- Zhou Q., Zhang J., Fu J., Shi J., Jiang G., 2007. Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. Anal Chim Acta; 14(606): 135–150. doi:10.1016/j.aca.2007.11.018
- Zollhöfer J.M., 1997. Quellen die unbekannten Biotope: erfassen, bewerten, schützen. Bristol-Schriftenreihe, Zürich, Bd. 6.
- Zollhöfer J.M., 1999. Spring habitats in northern Switzerland: Habitat heterogeneity, zoobentic communities, and colonization dynamics. Diss. ETH Nr.: 13209, Zürich.
- Zollhöfer J.M., Brunke A., Gonser T., 2000. A typology of springs in Switzerland by integrating habitat variables and fauna. Monographic studies. Supplement, 121: 349–376.

ATTACHMENTS

Annex 1. Toxic metals concentrations (mg/kg) in the different sediment fractions obtained from digested procedure

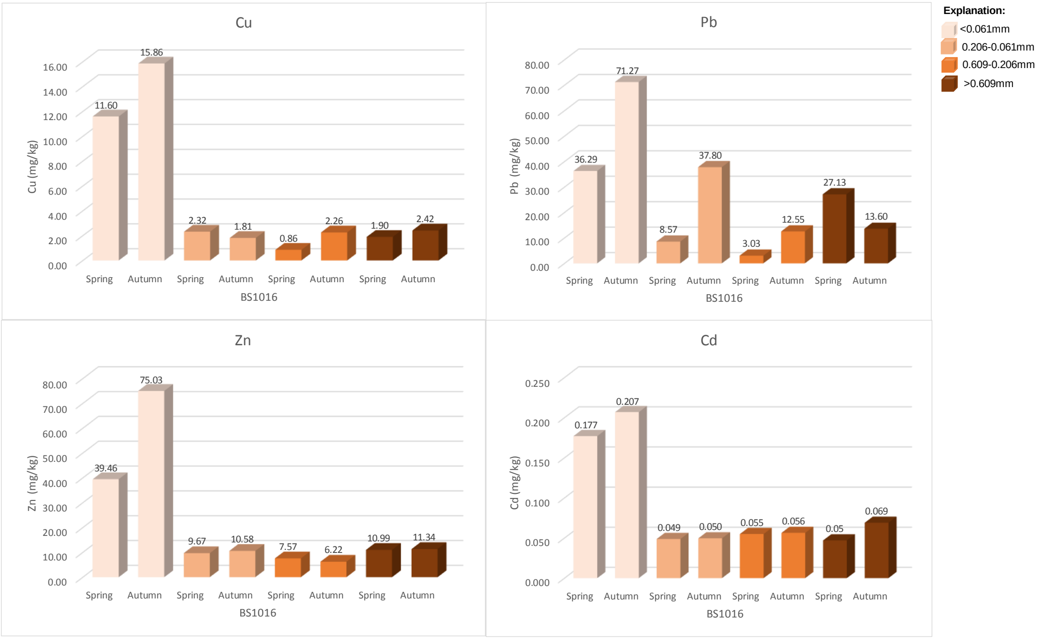
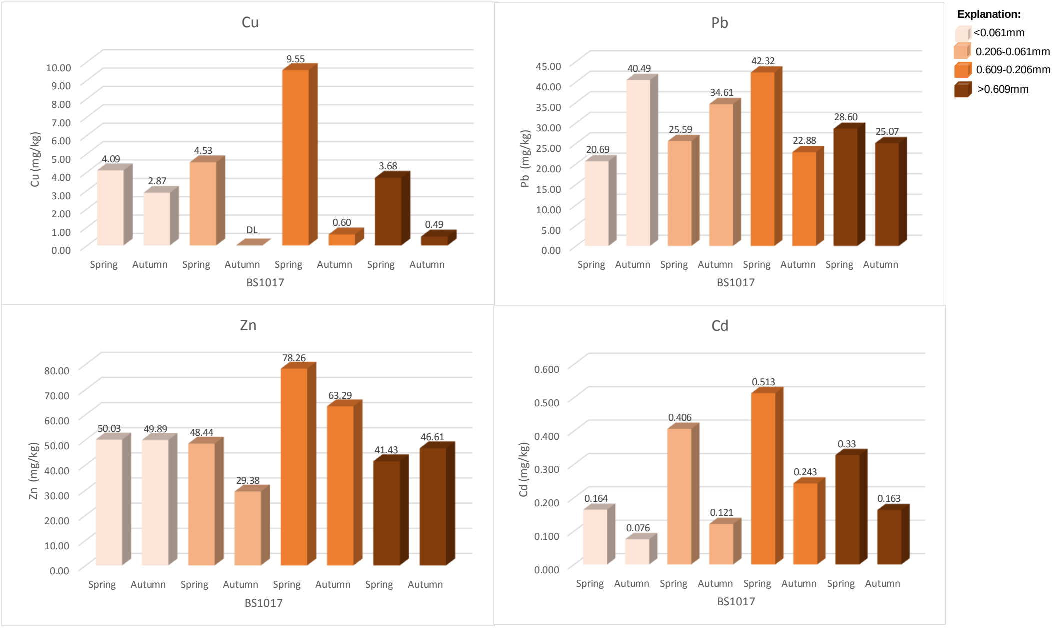


Fig. 1 BS1016



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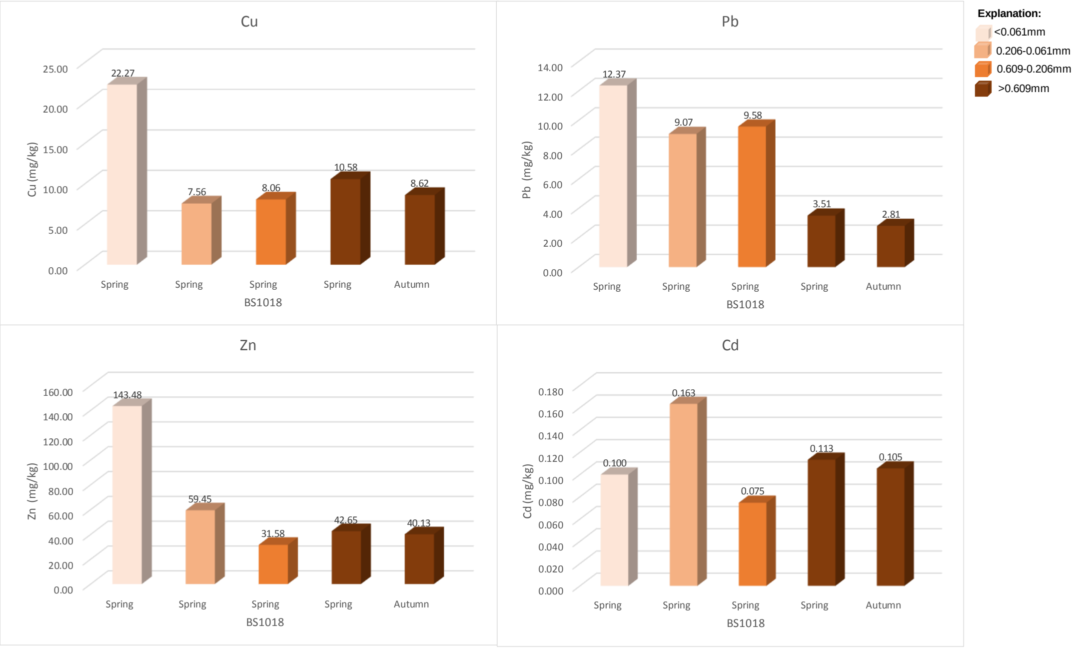

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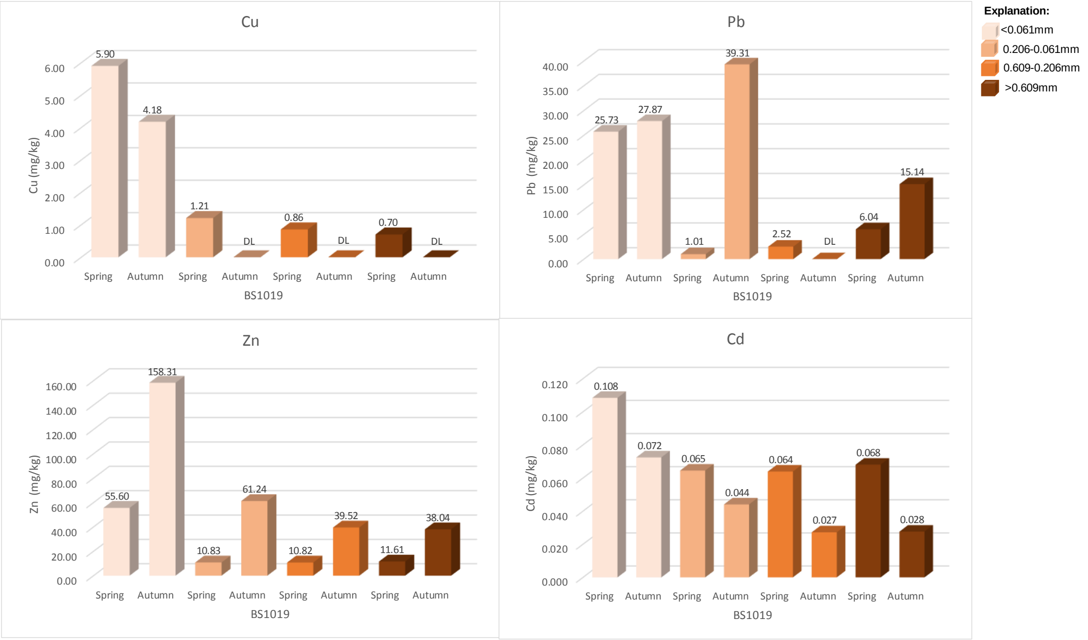

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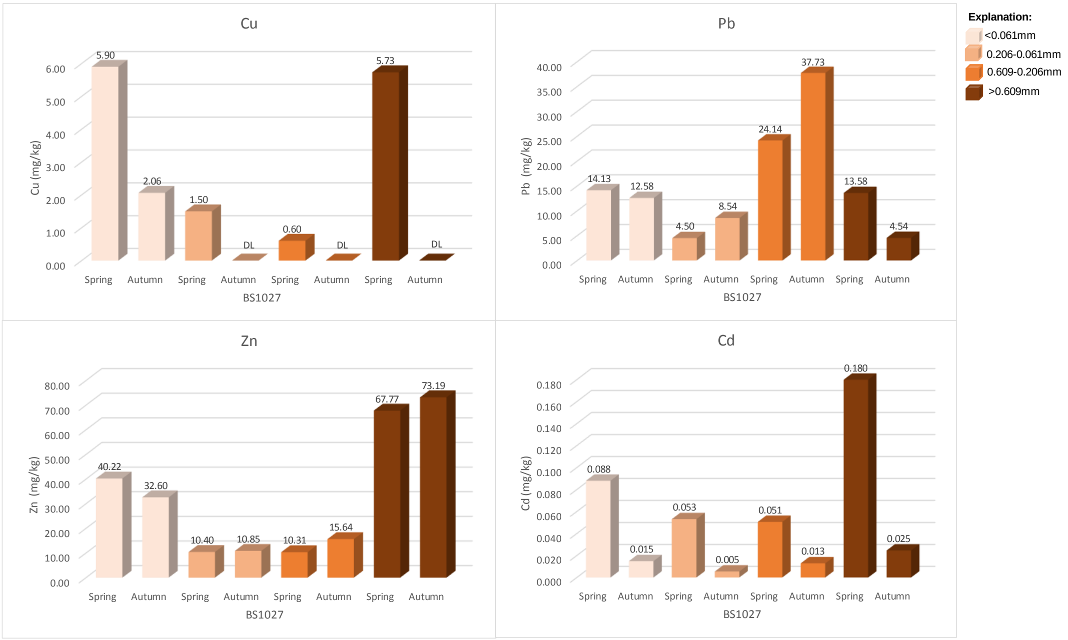

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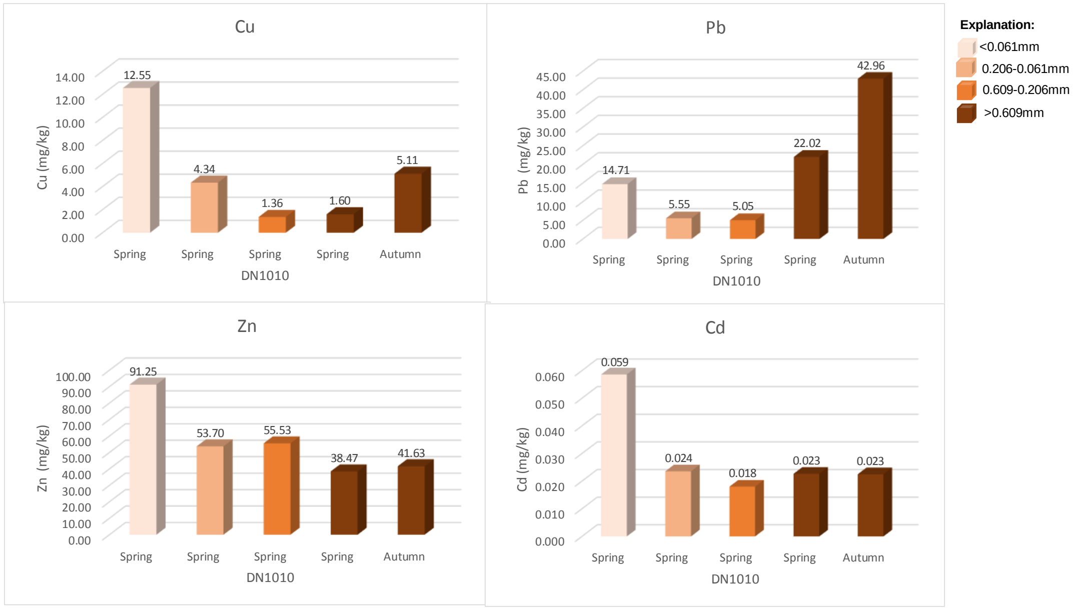

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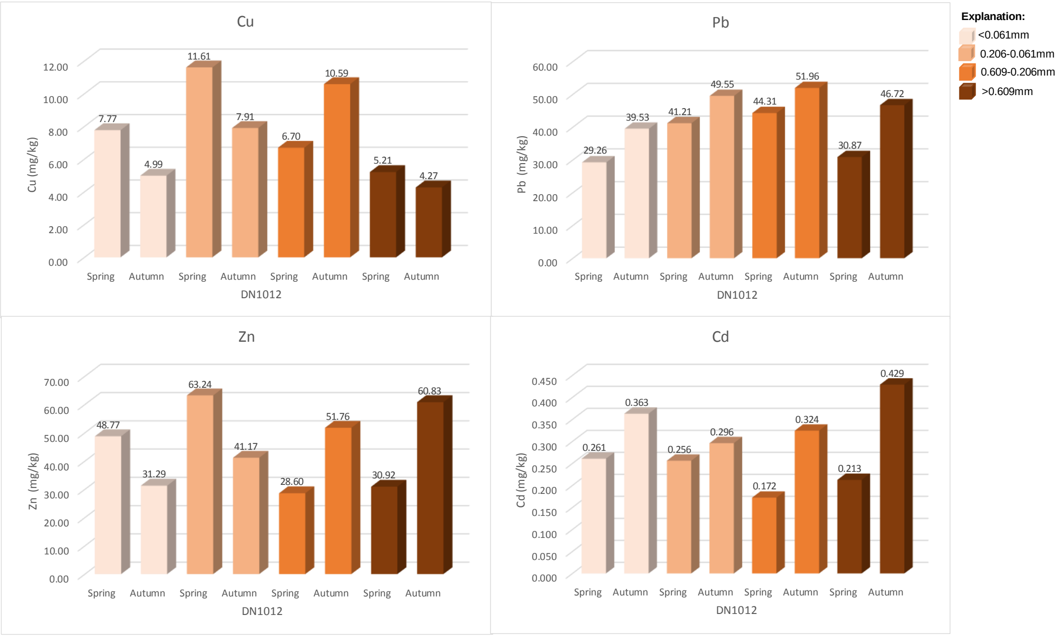

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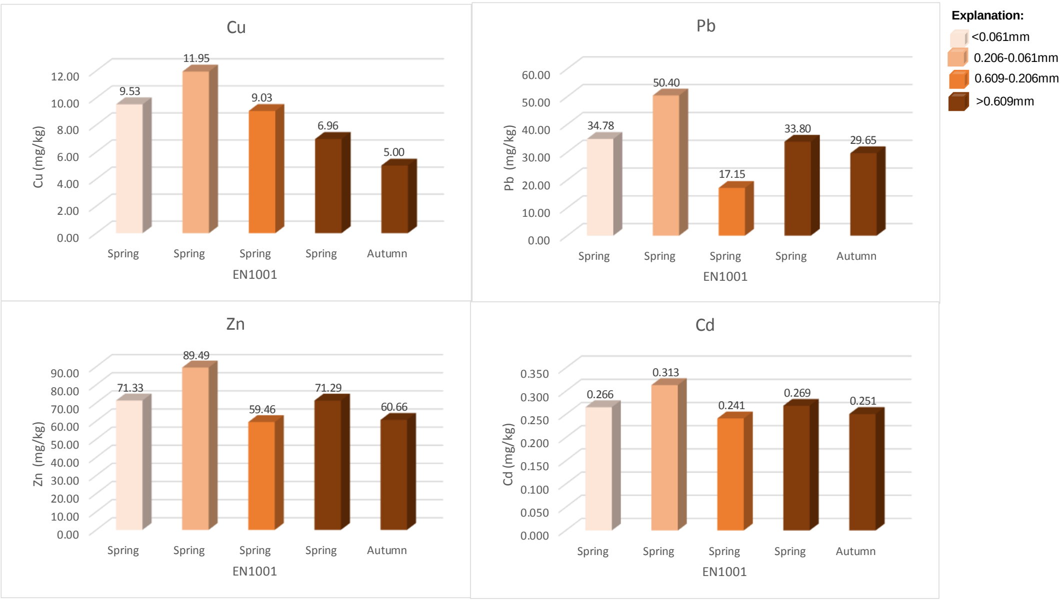
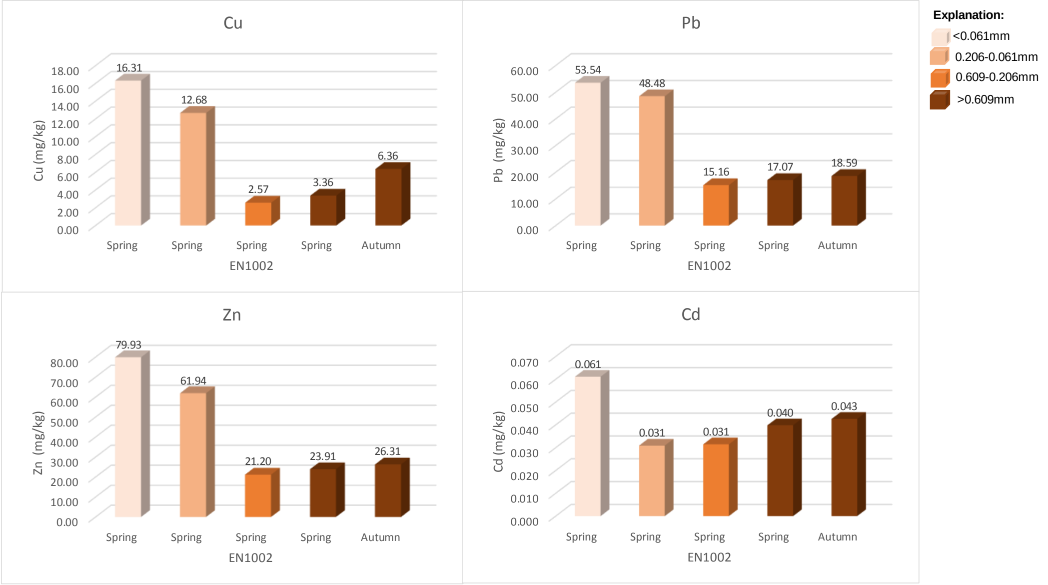


Fig. 8 EN1001



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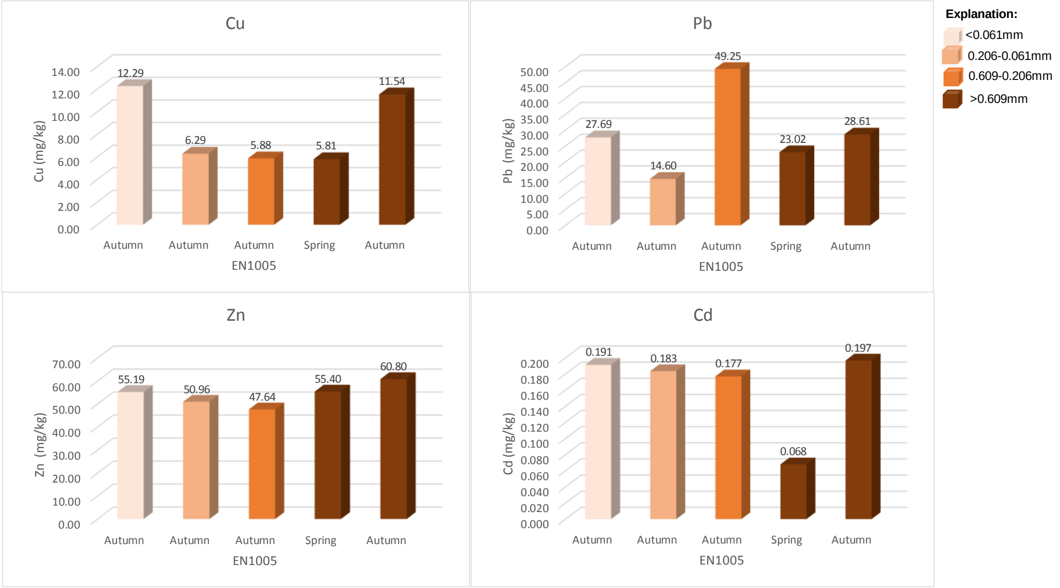


Fig. 10 EN1005

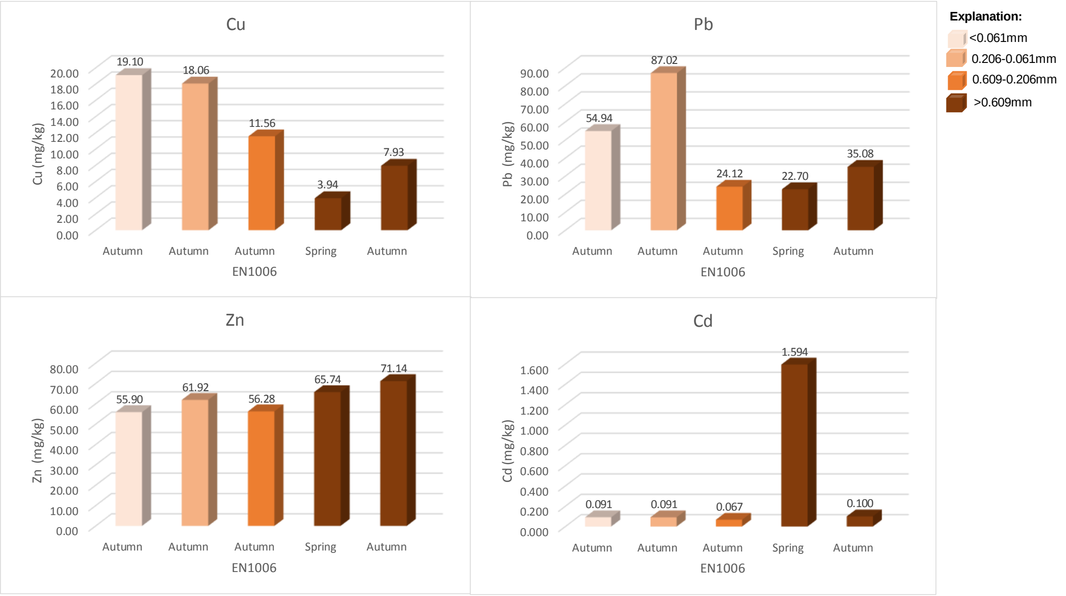
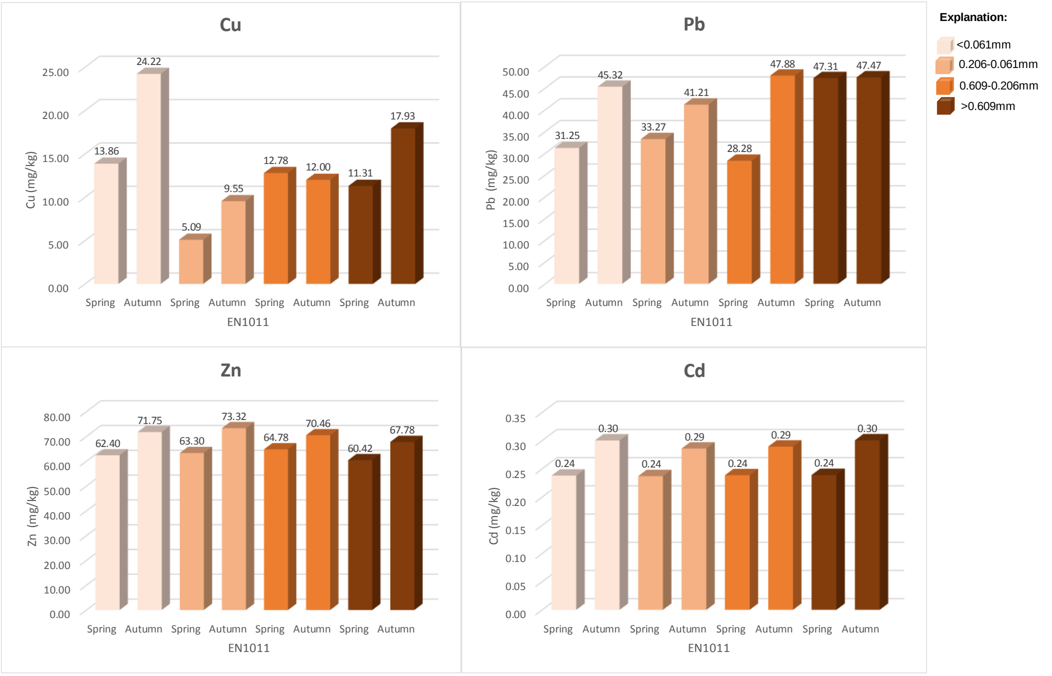


Fig. 11 EN1006



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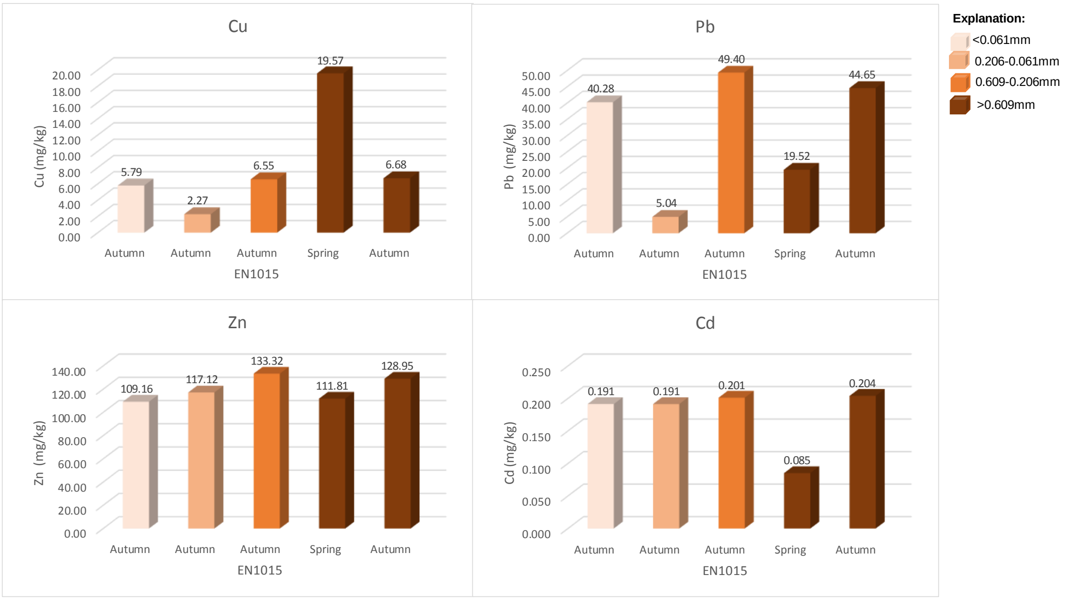

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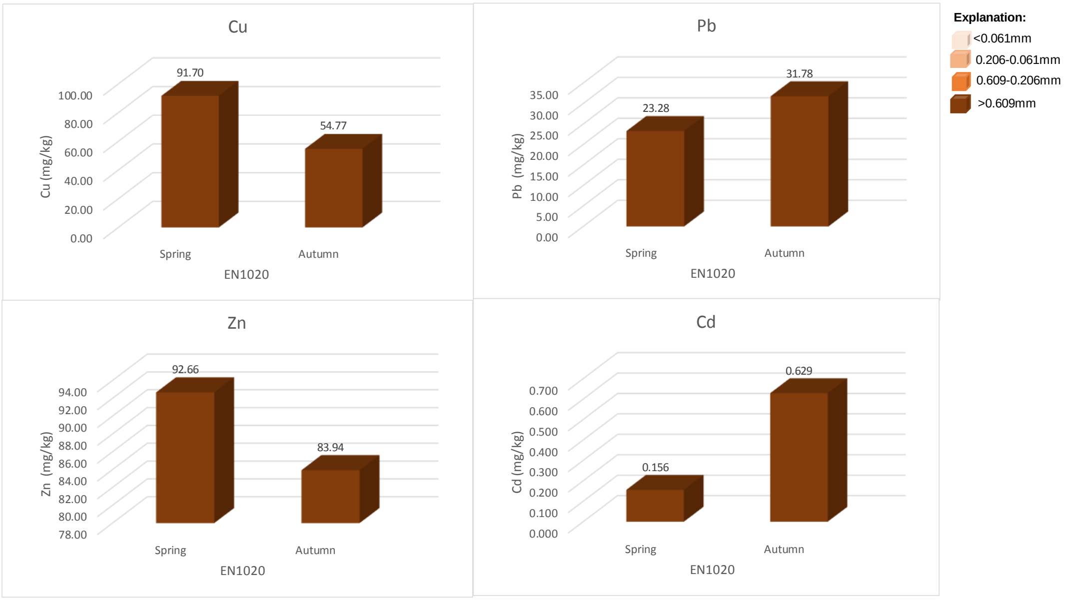

Fig. 14 EN1020

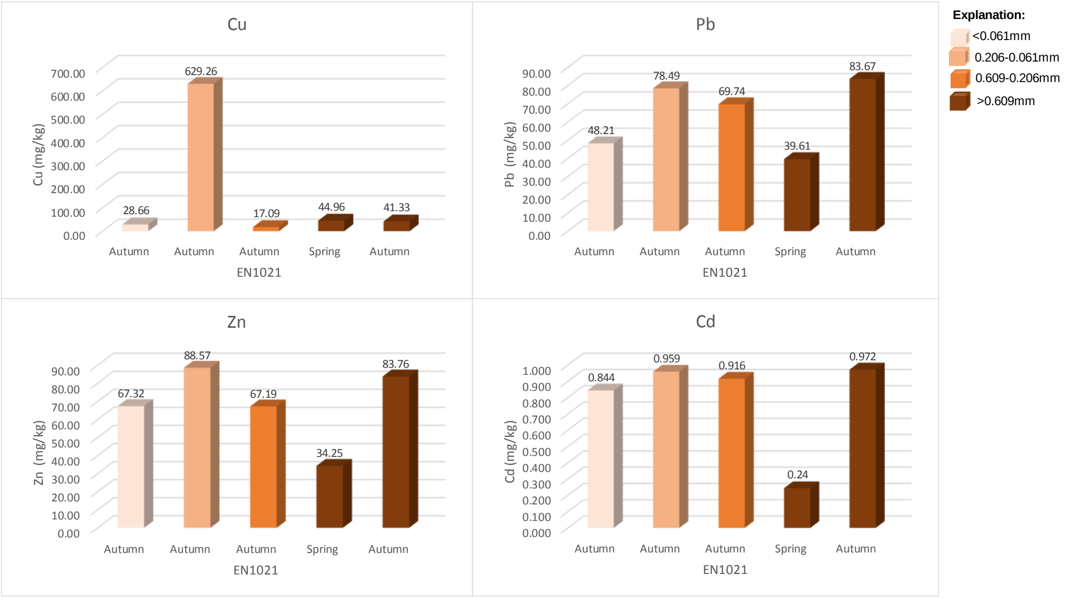

Fig. 15 EN1021

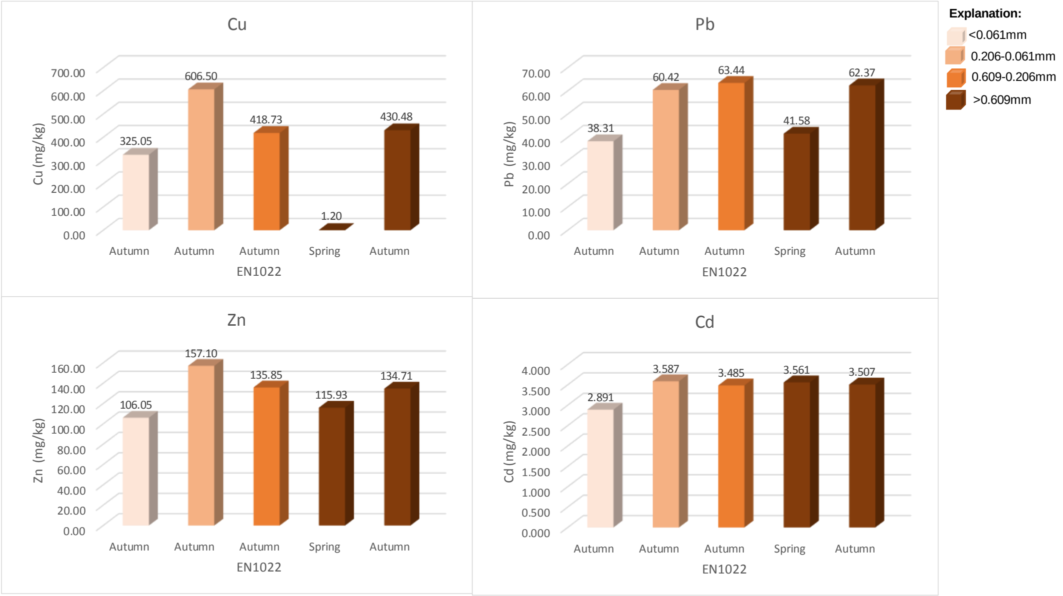

Fig. 16 EN1022

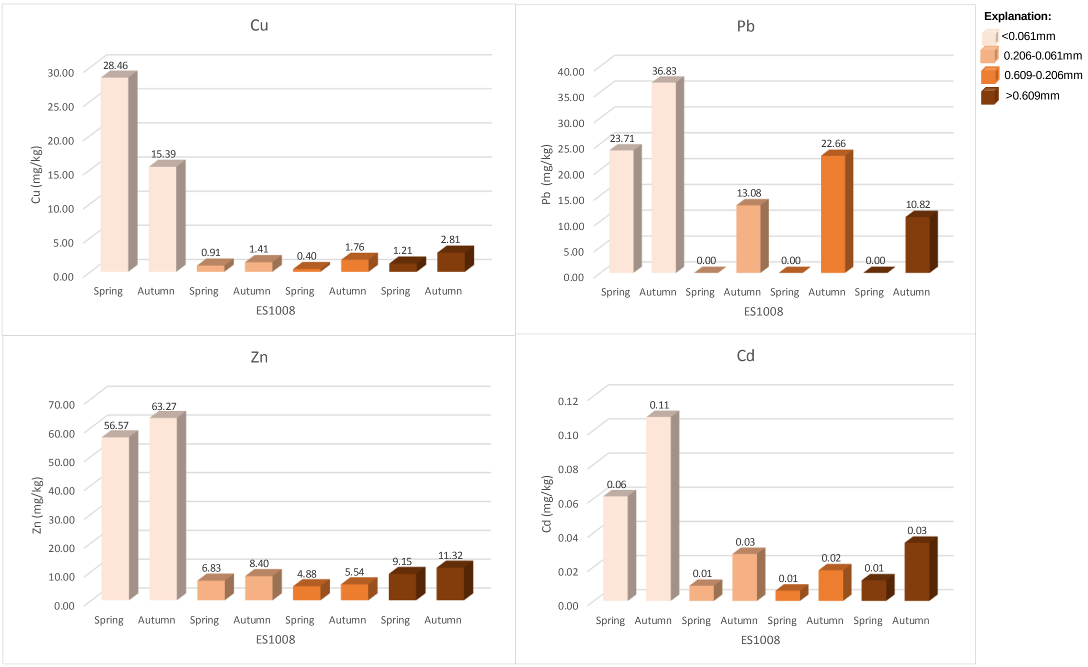

Fig. 17 ES1008

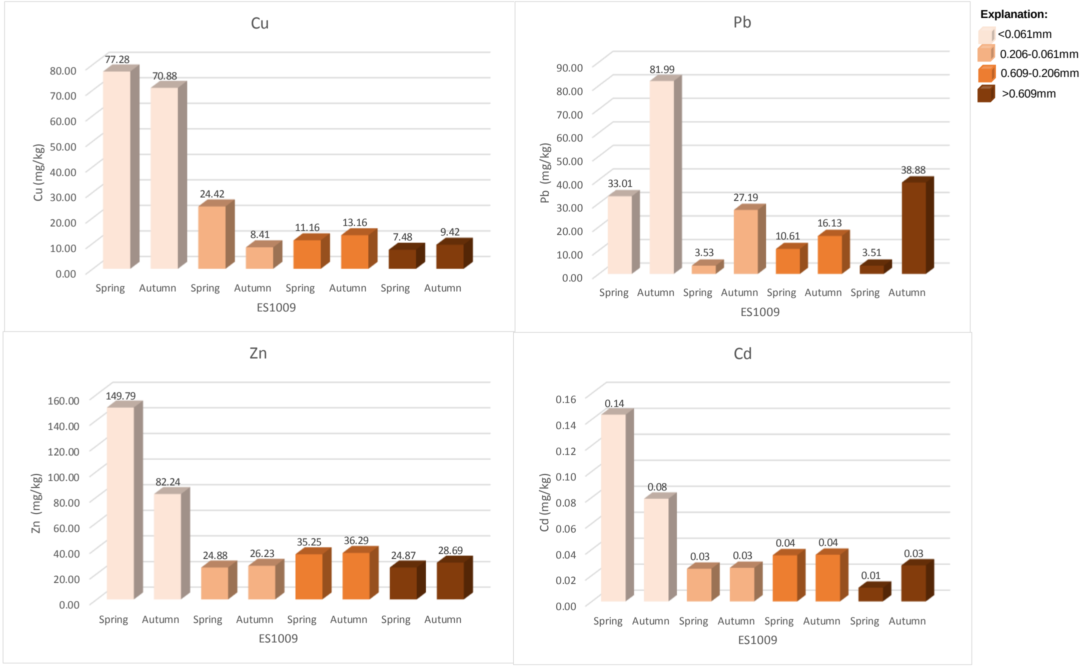

Fig. 18 ES1009

Annex 2. Biotic description and toxic metals concentration (mg/kg) in spring biota of sampling sites

				Concentration (mg/kg)			
Springs	**Description**	**Date of collection**	**Weight**	**Cu**	**Pb**	**Zn**	**Cd**
ES1014	Shrimps	24/10/2016	0.0342	36.26	1.87	122.57	0.37
ES1014	Caddisfly cases	24/10/2016	0.1028	4.86	3.53	59.18	2.07
ES1014	Mix(White worms, Bugs, Ceratopogonidae, Caddisflies larva)	24/10/2016	0.1005	8.46	3.60	142.82	2.14
ES1009	Shrimps	24/10/2016	0.0213	53.52	1.39	76.81	0.04
ES1009	Mix	24/10/2016	0.1918	18.04	0.18	78.61	0.01
ES1008	Shrimps	24/10/2016	0.1349	26.47	0.63	62.04	0.24
ES1008	Mix(Athericidae, White worms, Caddisflies cases)	24/10/2016	0.1645	8.21	1.20	120.13	0.68
EN1021	Shrimps	24/10/2016	0.0171	30.44	0.00	80.21	0.82
EN1021	Mix(White worms, Caddisflies cases, Bugs, small Mussels)	24/10/2016	0.0968	9.19	5.85	118.19	1.20
EN1011	Shrimps	24/10/2016	0.1557	31.09	0.33	53.80	0.52
EN1011	Mix(Caddisfly larva, Stratiomyidae)	24/10/2016	0.0661	6.51	0.64	115.10	1.70
EN1006	Shrimps	24/10/2016	0.1998	22.27	2.32	57.97	0.01
EN1006	Mix	24/10/2016	0.0568	8.63	4.25	179.08	0.07
EN1005	Shrimps	24/10/2016	0.1990	23.92	0.34	59.21	2.33
EN1005	Mix(Bugs, Ceratopogonidae, small Niphargus)	24/10/2016	0.0296	11.15	1.46	111.28	4.81
EN1005	Mix	24/10/2016	0.0927	8.74	0.13	77.73	1.30
EN1004	Mix, Nitěnky	24/10/2016	0.0921	15.42	6.43	171.40	5.99
EN1002	Mix(Worms, caddisflies larva, caddisflies cases)	24/10/2016	0.0645	8.99	6.93	46.82	0.93
EN1001	Mix(Shrimps, Trichoptera, Stratiomyidae)	24/10/2016	0.1901	7.52	2.69	108.84	2.17
DN1012	Mix(Aquatic worms, Caddisfly larva, Stratiomyidae)	31/10/2016	0.1980	17.32	3.80	75.37	2.94
DN1010	Shrimps	31/10/2016	0.0646	23.99	1.25	67.24	0.73
BS1027	Shrimps	26/10/2016	0.0252	30.56	0.88	81.59	0.17
BS1017	Mix(Worms, Stratiomyidae, Caddisfly larva)	25/10/2016	0.1805	6.48	2.03	94.24	0.96
BS1016	Mix(Shrimps, Bugs)	25/10/2016	0.1402	9.13	0.94	191.44	5.65

Annex 3. Toxic metals concentration (mg/L) in water of sampling sites

Spring	Sampling Date	Metals concentration (mg/L)			
		Cu	Zn	Cd	Pb
BS1016	October 24, 2016	0.0003	0.0034	0.0000	0.0000
BS1017	October 24, 2016	0.0008	0.0023	0.0000	0.0002
BS1019	October 26, 2016	0.0001	0.0014	0.0000	0.0001
BS1027	October 24, 2016	0.0001	0.0031	0.0000	0.0000
DN1012	October 14, 2016	0.0002	0.0035	0.0001	0.0001
EN1001	October 24, 2016	0.0003	0.0030	0.0000	0.0000
EN1002	October 24, 2016	0.0010	0.0014	0.0000	0.0000
EN1004	October 24, 2016	0.0001	0.0019	0.0001	0.0000
EN1005	October 24, 2016	0.0001	0.0010	0.0000	0.0000
EN1006	October 24, 2016	0.0002	0.0023	0.0000	0.0000
EN1011	October 24, 2016	0.0001	0.0002	0.0000	0.0000
EN1015	October 24, 2016	0.0011	0.0054	0.0001	0.0000
EN1021	October 24, 2016	0.0007	0.0066	0.0000	0.0001
EN1022	October 24, 2016	0.0015	0.0116	0.0004	0.0000
ES1008	October 24, 2016	0.0015	0.0009	0.0000	0.0001
ES1009	October 24, 2016	0.0003	0.0007	0.0000	0.0000
ES1014	October 24, 2016	0.0004	0.0011	0.0000	0.0001

Annex 4. Metal contents (mg.km^{-1}) in digested sediment fractions collected from in the Lužické mountains springs, Liberec.

Site	Site	Metal	< 0.061 mm		0.206 - 0.061 mm		0.609 - 0.206 mm		> 0.609 mm (Total fraction)	
number	name	contents	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
2	BS1016	Cd	0.18	0.21	0.05	0.05	0.06	0.06	0.05	0.07
		Zn	39.46	75.03	9.67	10.58	7.57	6.22	10.99	11.34
		Cu	11.60	15.86	2.32	1.81	0.86	2.26	1.90	2.42
		Pb	36.29	71.27	8.57	37.80	3.03	12.55	27.13	13.60
3	BS1017	Cd	0.16	0.08	0.41	0.12	0.51	0.24	0.33	0.16
		Zn	50.03	49.89	48.44	29.38	78.26	63.29	41.43	46.61
		Cu	4.09	2.87	4.53	DL	9.55	0.60	3.68	0.49
		Pb	20.69	40.49	25.59	34.61	42.32	22.88	28.60	25.07
4	BS1018	Cd	0.10	-	0.16	-	0.07	-	0.11	0.11
		Zn	143.48	-	59.45	-	31.58	-	42.65	40.13
		Cu	22.27	-	7.56	-	8.06	-	10.58	8.62
		Pb	12.37	-	9.07	-	9.58	-	3.51	2.81
5	BS1019	Cd	0.11	0.07	0.06	0.04	0.06	0.03	0.07	0.03
		Zn	55.60	158.31	10.83	61.24	10.82	39.52	11.61	38.04
		Cu	5.90	4.18	1.21	DL	0.86	DL	0.70	DL
		Pb	25.73	27.87	1.01	39.31	2.52	DL	6.04	15.14
6	BS1027	Cd	0.09	0.01	0.05	0.01	0.05	0.01	0.18	0.02
		Zn	40.22	32.60	10.40	10.85	10.31	15.64	67.77	73.19
		Cu	5.90	2.06	1.50	DL	0.60	DL	5.73	DL
		Pb	14.13	12.58	4.50	8.54	24.14	37.73	13.58	4.54
7	DN1010	Cd	0.06	-	0.02	-	0.02	-	0.02	0.02
		Zn	91.25	-	53.70	-	55.53	-	38.47	41.63
		Cu	12.55	-	4.34	-	1.36	-	1.60	5.11
		Pb	14.71	-	5.55	-	5.05	-	22.02	42.96
8	DN1012	Cd	0.26	0.36	0.26	0.30	0.17	0.32	0.21	0.43
		Zn	48.77	31.29	63.24	41.17	28.60	51.76	30.92	60.83
		Cu	7.77	4.99	11.61	7.91	6.70	10.59	5.21	4.27
		Pb	29.26	39.53	41.21	49.55	44.31	51.96	30.87	46.72
10	EN1001	Cd	0.27	-	0.31	-	0.24	-	0.27	0.25
		Zn	71.33	-	89.49	-	59.46	-	71.29	60.66
		Cu	9.53	-	11.95	-	9.03	-	6.96	5.00
		Pb	34.78	-	50.40	-	17.15	-	33.80	29.65
11	EN1002	Cd	0.06	-	0.03	-	0.03	-	0.04	0.04
		Zn	79.93	-	61.94	-	21.20	-	23.91	26.31
		Cu	16.31	-	12.68	-	2.57	-	3.36	6.36
		Pb	53.54	-	48.48	-	15.16	-	17.07	18.59
12	EN1004	Cd	0.09	0.30	0.23	0.07	0.05	0.15	0.08	0.25
		Zn	78.51	88.50	32.83	31.42	13.08	13.56	33.31	37.16
		Cu	10.78	10.04	4.64	6.50	2.93	5.95	7.24	5.72
		Pb	34.24	66.60	19.66	25.18	6.05	53.43	18.09	58.68
13	EN1005	Cd	-	0.19	-	0.18	-	0.18	0.07	0.20
		Zn	-	55.19	-	50.96	-	47.64	55.40	60.80
		Cu	-	12.29	-	6.29	-	5.88	5.81	11.54
		Pb	-	27.69	-	14.60	-	49.25	23.02	28.61
14	EN1006	Cd	-	0.09	-	0.09	-	0.07	1.59	0.10
		Zn	-	55.90	-	61.92	-	56.28	65.74	71.14
		Cu	-	19.10	-	18.06	-	11.56	3.94	7.93
		Pb	-	54.94	-	87.02	-	24.12	22.70	35.08
15	EN1011	Cd	0.24	0.30	0.24	0.29	0.24	0.29	0.24	0.30
		Zn	62.40	71.75	63.30	73.32	64.78	70.46	60.42	67.78
		Cu	13.86	24.22	5.09	9.55	12.78	12.00	11.31	17.93
		Pb	31.25	45.32	33.27	41.21	28.28	47.88	47.31	47.47

Annex 4. (*continued*)

Site number	Site name	Metal contents	<0.061 mm		0.206-0.061 mm		0.609-0.206 mm		> 0.609 mm (Total fraction)	
			Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
16	EN1015	Cd	-	0.19	-	0.19	-	0.20	0.09	0.20
		Zn	-	109.16	-	117.12	-	133.32	111.81	128.95
		Cu	-	5.79	-	2.27	-	6.55	19.57	6.68
		Pb	-	40.28	-	5.04	-	49.40	19.52	44.65
17	EN1020	Cd	-	-	-	-	-	-	0.16	0.63
		Zn	-	-	-	-	-	-	92.66	83.94
		Cu	-	-	-	-	-	-	91.70	54.77
		Pb	-	-	-	-	-	-	23.28	31.78
18	EN1021	Cd	-	0.84	-	0.96	-	0.92	0.24	0.97
		Zn	-	67.32	-	88.57	-	67.19	34.25	83.76
		Cu	-	28.66	-	629.26	-	17.09	44.96	41.33
		Pb	-	48.21	-	78.49	-	69.74	39.61	83.67
19	EN1022	Cd	-	2.89	-	3.59	-	3.49	3.56	3.51
		Zn	-	106.05	-	157.10	-	135.85	115.93	134.71
		Cu	-	325.05	-	606.50	-	418.73	1.20	430.48
		Pb	-	38.31	-	60.42	-	63.44	41.58	62.37
20	ES1008	Cd	0.06	0.11	0.01	0.03	0.01	0.02	0.01	0.03
		Zn	56.57	63.27	6.83	8.40	4.88	5.54	9.15	11.32
		Cu	28.46	15.39	0.91	1.41	0.40	1.76	1.21	2.81
		Pb	23.71	36.83	0.00	13.08	DL	22.66	0.00	10.82
21	ES1009	Cd	0.14	0.08	0.03	0.03	0.04	0.04	0.01	0.03
		Zn	149.79	82.24	24.88	26.23	35.25	36.29	24.87	28.69
		Cu	77.28	70.88	24.42	8.41	11.16	13.16	7.48	9.42
		Pb	33.01	81.99	3.53	27.19	10.61	16.13	3.51	38.88
22	ES1014	Cd	0.84	0.47	1.00	0.54	0.86	0.49	0.94	0.74
		Zn	60.39	37.68	90.86	47.68	71.97	40.95	80.85	62.68
		Cu	10.71	6.97	0.39	5.17	10.93	1.23	10.75	3.02
		Pb	26.52	34.64	24.61	33.99	27.56	17.71	24.65	45.96

Annex 5. The partition of metal contents (mg.km^{-1}) in the fractions obtained by the BCR sequential extraction technique in the Lužické mountains springs, Liberec.

Site number	Description	Metal contents	1 Mobile/exchangeable	2 Reducible	3 Oxidizable	4 Residuals	Sum (1-4 fractions) (mg/kg)
2	BS1016	Cd	0.01	DL	0.01	DL	0.02
		Zn	2.72	6.03	4.83	2.96	16.54
		Cu	1.01	1.02	2.12	0.30	4.45
		Pb	DL	0.30	3.60	0.82	4.72
3	BS1017	Cd	0.07	DL	0.02	DL	0.08
		Zn	8.52	13.82	7.91	9.54	39.79
		Cu	0.28	1.21	0.97	0.43	2.89
		Pb	0.63	0.63	1.63	0.21	3.09
5	BS1019	Cd	0.01	0.00	0.01	0.00	0.02
		Zn	3.68	2.94	15.51	10.32	32.45
		Cu	0.19	0.26	2.32	3.75	6.53
		Pb	0.59	0.09	1.68	5.00	7.36
6	BS1027	Cd	0.01	DL	0.01	0.03	0.05
		Zn	3.19	9.36	5.43	4.11	22.08
		Cu	0.18	0.65	0.67	0.29	1.80
		Pb	0.21	0.09	0.44	8.50	9.24
7	DN1010	Cd	0.06	DL	0.01	DL	0.07
		Zn	4.21	7.98	14.97	5.90	33.06
		Cu	0.55	0.76	1.76	0.43	3.50
		Pb	0.42	0.57	1.52	0.40	2.90
8	DN1012	Cd	0.13	0.02	0.03	0.01	0.19
		Zn	6.52	11.74	15.69	15.82	49.77
		Cu	0.30	1.26	4.56	0.75	6.88
		Pb	0.42	12.14	3.54	9.00	25.10
11	EN1002	Cd	0.05	DL	0.02	0.01	0.08
		Zn	6.36	13.40	26.63	10.52	56.92
		Cu	0.53	3.80	8.32	1.13	13.78
		Pb	1.63	20.84	5.60	5.00	33.07
12	EN1004	Cd	0.07	0.00	0.00	0.00	0.08
		Zn	13.32	7.60	6.42	2.09	29.41
		Cu	0.58	2.12	0.46	0.24	3.41
		Pb	0.92	1.13	0.26	6.50	8.81
13	EN1005	Cd	0.02	0.00	0.06	0.01	0.08
		Zn	21.27	12.76	16.95	7.90	58.88
		Cu	0.61	2.40	2.52	2.52	8.05
		Pb	0.88	0.64	2.35	0.25	4.12
14	EN1006	Cd	0.05	0.01	0.08	0.06	0.20
		Zn	13.41	12.50	25.96	34.94	86.80
		Cu	1.09	6.10	7.78	6.87	21.84
		Pb	2.63	0.93	7.21	5.25	16.02

Annex 5. (*continued*)

Site number	Description	Metal contents	1 Mobile/exchangeable	2 Reducible	3 Oxidizable	4 Residuals	Sum (1-4 fractions) (mg/kg)
15	EN1011	Cd	0.05	0.02	0.03	0.02	0.12
		Zn	6.35	14.34	22.46	24.71	67.87
		Cu	0.32	2.50	5.75	3.30	11.87
		Pb	0.42	0.84	7.98	7.49	16.73
16	EN1015	Cd	0.07	0.01	0.02	0.02	0.12
		Zn	14.61	12.34	27.98	10.67	65.61
		Cu	0.32	1.96	6.68	0.90	9.86
		Pb	0.80	16.66	7.62	9.50	34.58
17	EN1020	Cd	0.07	0.14	0.13	0.09	0.43
		Zn	22.88	43.66	36.49	27.83	130.87
		Cu	0.74	2.48	28.48	12.55	44.25
		Pb	0.34	0.30	6.00	11.00	17.64
18	EN1021	Cd	0.12	0.19	0.11	0.05	0.48
		Zn	14.89	25.62	24.00	34.78	99.29
		Cu	0.61	2.11	39.48	9.05	51.25
		Pb	0.38	0.64	7.55	17.00	25.57
19	EN1022	Cd	2.73	2.28	0.28	0.20	5.49
		Zn	23.75	19.69	27.03	47.43	117.90
		Cu	73.00	188.92	66.72	27.25	355.89
		Pb	0.24	0.75	10.79	12.50	24.27
21	ES1009	Cd	0.00	0.00	0.01	0.01	0.02
		Zn	11.84	15.08	10.84	5.78	43.54
		Cu	0.99	5.12	4.12	2.07	12.30
		Pb	0.42	0.18	0.48	0.66	1.74
22	ES1014	Cd	0.13	0.05	0.03	0.01	0.22
		Zn	14.73	23.37	39.08	12.98	90.16
		Cu	0.12	1.16	5.67	0.00	6.96
		Pb	0.10	0.21	6.78	5.00	12.08

Annex 6. Analyses of covariance between toxic metals concentration (mg/kg) in biota and sediments, using linear models (LM)

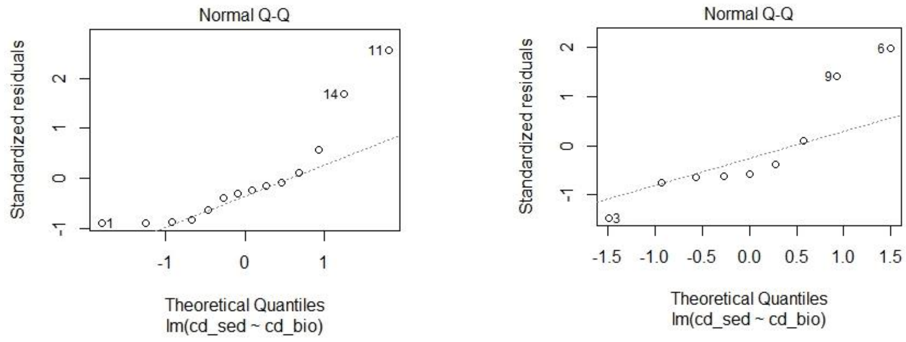

Fig. 19 Statistically significant covariances among Cd concentration (mg/kg) in sediments and biota (Left - mix, right - shrimps).

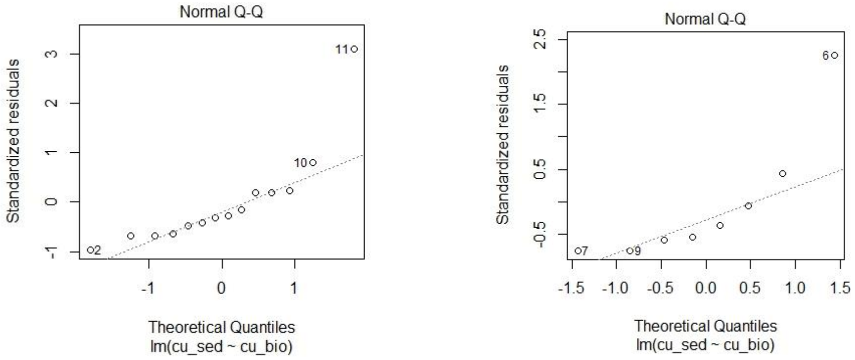

Fig. 20 Statistically significant covariances among Cu concentration (mg/kg) in sediments and biota (Left - mix, right - shrimps).

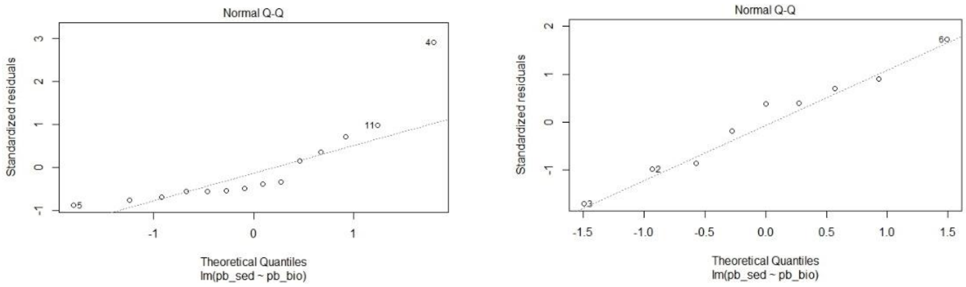

Fig. 21 Statistically significant covariances among Pb concentration (mg/kg) in sediments and biota (Left - mix, right - shrimps)

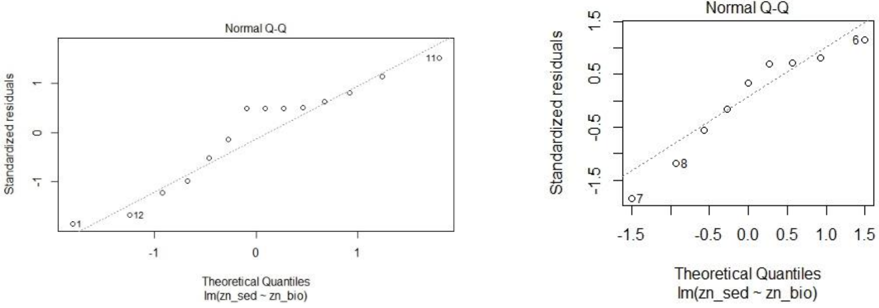

Fig. 22 Statistically significant covariances among Zn concentration (mg/kg) in sediments and biota (Left - mix, right - shrimps).