Journal of Elementology ISSN 1644-2296

Senze M., Kowalska-Góralska M. 2020.
Evaluation of the bioaccumulation of metals in submerged plants of the Verdon River and Lake Sainte-Croix (France) – preliminary research.
J. Elem., 25(1): 297-314. DOI: 10.5601/jelem.2019.24.4.1884

RECEIVED: 27 June 2019
ACCEPTED: 24 October 2019

ORIGINAL PAPER

EVALUATION OF THE BIOACCUMULATION OF METALS IN SUBMERGED PLANTS OF THE VERDON RIVER AND LAKE SAINTE-CROIX (FRANCE) – PRELIMINARY RESEARCH*

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Abstract

The objective of the study has been to evaluate aquatic environment quality in Sainte-Croix Reservoir (Lake Sainte-Croix) and the Verdon River that feeds the reservoir as well as water of the Verdon River discharged from the reservoir. The evaluation was based on the accumulation of heavy metals tested in aquatic plants. The study material were water samples and aquatic plants. pH, electrolytic conductivity and metals (Cu, Cd, Pb, Zn, Ni) were determined in water. Only submerged plants were collected for testing, which was a purposeful choice because the objective was to test pollutants in the plants whose whole surface was in contact with water. The flora was dominated by the genus *Potamogeton*. Metal concentrations in water occurred in the following order: Pb<Cd<Ni<Cu<Zn, and in plants: Cd<Pb<Cu<Ni<Zn. The accumulation of metals according to the bioaccumulation factor (BCF) was as follows: Cu<Zn<Cd<Pb<Ni. Metal pollution indices (MPIs) for water in the test sites were: 2<1<6<5<3<4<7, and for aquatic plants: 1<6<7<3<5<2<4. These findings are typical of a number of reservoirs, and confirm their retention role. Water that feeds the reservoir has lower metal content than that in the reservoir, and the highest levels are found in the water discharged from the reservoir. A general tendency in the accumulation of metals in the reservoir is seen. It can be concluded that the reservoir may play a positive role owing to its potential accumulation capacity. Differences between the sites can be noted for all metals. Correlations between the tested parameters were determined, and the most statistically significant correlations were found between the plant species and accumulation of all the metals tested (except Zn), which was also confirmed by PCA. No statistically significant correlations were found between the concent of metals in water and in plants. Further research including analyses of the content of metals in the sediments, as well as in the immediate vicinity of the reservoir, i.e. the soil and other components of the catchment basin, planned to be carried out in the subsequent series of studies, is expected to resolve the above problem.

Keywords: heavy metals, Verdon River, Lake Sainte-Croix, hydromacrophytes, water, bioconcentration, MPI (metal pollution index).

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* Research projects as part of statutory research.

INTRODUCTION

Dam reservoirs are created by fencing a river valley with artificial dams (stone, concrete, earth). These are special places, constructed by man for a specific purpose. Their role is mainly to retain water, but they can also serve to generate power and as recreational sites. An additional, but equally important task of dam reservoirs is to gather pollutants from the catchment area. They are most often anthropogenic in origin, derived from municipal, economic and agricultural activities, but some are a result from progressing natural processes. They come from point or nonpoint sources of pollution, and their level in the water environment reflects what happens in the catchment area (SZALIŃSKA et al. 2010). The geological structure of the catchment and the prevailing climatic conditions are particularly important. The construction of a reservoir changes the hydrological system in the catchment, as a large water area appears in the environment, which, apart from inducing local climate changes, becomes a habitat for living organisms. The river on which a reservoir is built also changes its character. The construction of a dam becomes the cause of changes in its course, chemical composition and species composition of organisms living there (DOYLE et al. 2003, JONCZAK, PARZYCH 2019).

The construction of an artificial water body is often associated with significant changes in the land cover. Forested areas, meadows, cultivated fields, but also buildings are eliminated.

Preliminary works, which often last for several years, change the natural landscape. Several years after filling, the reservoir reaches its typical ecological state and the conditions prevailing there can be considered as typical of this place. The reservoir called Lake Sainte-Croix is already stabilized, and now only natural and human activity can cause subsequent changes in this water body. The diversity of substances in a reservoir most often referred to as pollution mirrors the environment. Substances imported with the rivers feeding a reservoir and originating from its immediate vicinity are incorporated into its structure, i.e. water, bottom sediments, plants and aquatic animals. These biotic and abiotic components are a good indicator of the level of pollution in the water environment. Heavy metals are mentioned among the pollutants whose presence in water is undesirable. Their level determined in aquatic plants may be an indicator employed to assess the quality of an aquatic environment (TEODOROVIC et al. 2000, QINGJIE G., JUN D. 2008, RAJFUR et al. 2010, HOWARD, OLULU 2012, MOHIUDDIN et al. 2012). Water plants growing in flowing and standing water reservoirs are an excellent indicator of changes occurring within the water environment. Rooted plants have direct contact with water, but also absorb building components from the bottom sediment. Therefore, their composition will depict the composition of water and sediment. On the other hand, floating plants that are not rooted have no contact with the bottom sediment, and usually float freely in the water with their entire surface submerged in water. They can therefore be transported downstream and absorb components from the water through their entire surface (RAJFUR et al. 2010, MOHIUDDIN et al. 2012).

Research on standing and flowing water reservoirs in France has been carried out for many years. The wide scope of these studies includes the rivers of southern France, for example the catchment area of the Durance River (flowing to the Rhône) and the Verdon River feeding it, as well as the Lake Sainte-Croix dam reservoir built on it. The study of these waters focuses on both assessment of the physical, chemical and bacteriological properties of the water and observation of aquatic fauna and flora. Also, it seeks to answer questions associated with climatology and hydroengineering. Thus, the above research covers comprehensively the environment of flowing and standing waters of this region of France (CHAMPEAU et al. 1982, BIN MOLÉ et al. 1986, POUCHER, SALENÇON 1990, CAZAUBON, GIUDICELLI 1999, FAYOLLE et al. 1999, BERTRAND et al. 2001, BERTRAND et al. 2003).

Lake Sainte-Croix is an oligotrophic reservoir (POUCHER, SALENÇON 1990). The catchment does not contain any significant sources of contamination with compounds of anthropogenic origin. In addition to small towns, the main catchment area is made up of upland and mountainous areas. The location of the research stations is closely related to the purpose of the work, and was motivated by the desire to learn about the content of metals in the Verdon River and the dam reservoir located there, as well as to determine the variability of their levels along with the direction of water flow. Moreover, it was decided to explore the impact of human activity on the quality of the water environment of the reservoir. Therefore, the choice of research stations on the Verdon River, i.e. selecting sites located below the towns through which the river flows, was not accidental. The focus of attention was on areas with potentially increased human impact. Thus, on the north-western shore, has a relatively straight shhoreline compared to the opposite side, the research was concentrated on areas with potentially stronger human impact.

MATERIAL AND METHODS

Study area

The study area was located between N43°44′13.7611″ - N43°49′41.4123″ and E6°7′ 56.4325″ - E6°27′52.3416″ (Figure 1). The Verdon River is a tributary of the Durance River in France. Its sources are located 2819 m a.s.l. in the South-Western Alps. Its length is 175 km. The river is heavily regulated, with five dams and five reservoirs (Lake Castillon, Lake Chaudanne, Lake Sainte-Croix, Retenue de Quinson and Lake d’Esparron) built on it in 1923-1975. These reservoirs serve water retention, energy generation

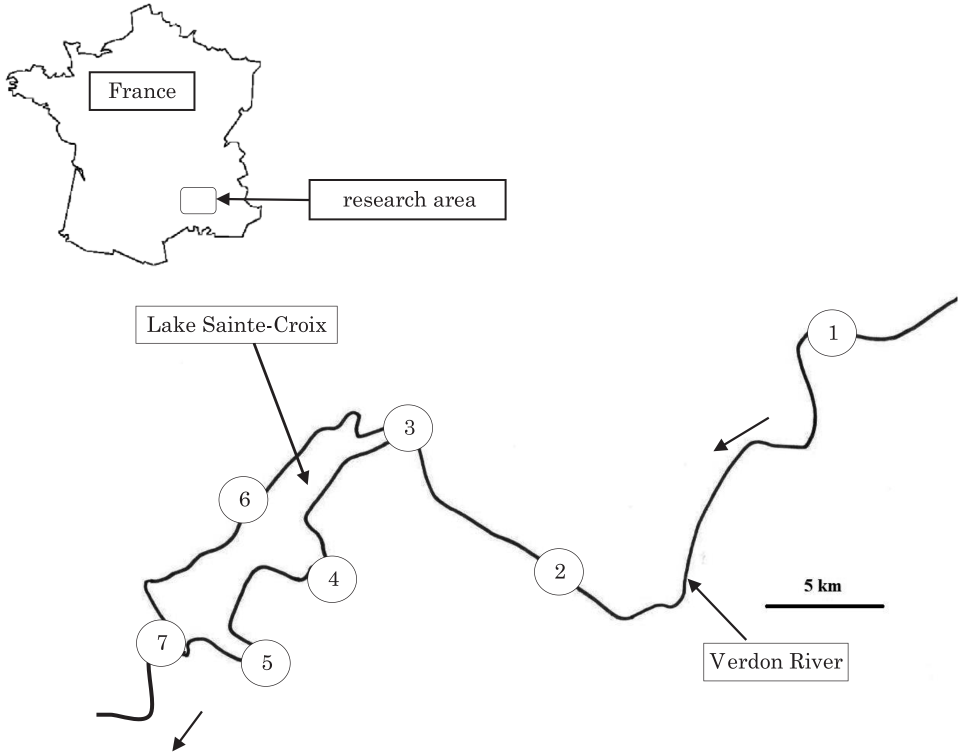


Fig. 1. Location of sampling points on the Verdon River and Lake Sainte-Croix

and recreational purposes (Nicod 1974, Brun et al. 1990, Bertrand et al. 2001, Warner 2012, Branche 2016).

Lake Sainte-Croix was built between 1971-1974. The concept that involved flooding the Salles Valley and creating a reservoir of the Verdon was devised as early as in 1908, but it was not implemented until 1968, when the French government funded it. The reservoir is the third largest one in France. Its area is 22 km^2 (10 km long and 2 km wide), and its capacity equals 761 million m^3. It has an arch iron dam, which is 94 m high, and the base measures 7.5 m in width while the crest is 3 m broad. It stands at the entrance to the Beaudinard gorges. There is a power plant at the dam (150 mln kWh per year). The water reservoir also serves as a fire-fighting tank for the surrounding area, and the water from it is drawn by fire-fighting planes. The Verdon River forms one of the deepest gorges in Europe, situated at a 21 km section upstream off the reservoir. Owing to its natural values, it is a suitable place for rafting, while the reservoir is good for sailing and windsurfing, which attracts tourists (Nicod 1974, Brun et al. 1990, Bertrand et al. 2001, Branche 2016).

Sampling collection and analysis

Both in the river and in the reservoir, the water plant associations are poor in terms of the number of species and their abundance. In most cases, if there are any plant species, the majority of them are submerged. Due to unfavourable rooting conditions (inconvenient substrate), there is no belt of helorophytes. This is caused by the geological structure, mostly composed of limestone, which allowed the creation of the Verdon River canyon. The shoreline zone of the river and Lake Sainte-Croix are poor in aquatic plants. The river banks are typically mountainous, mostly rocky, steep and sharp, while in some places the river overflows and there are only stones and sand as the shore material. The shores of the reservoir look similar. Although as steep as in the river banks, and the shallow part of the reservoir can be used for recreation, most of the shoreline material consists of fine stones, sand and partly rock.

The study material comprised only samples of water and aquatic plants taken from the Verdon River and from the littoral zone of Lake Sainte-Croix (Table 1, Figure 1). Bottom sediments were not collected for technical

Table 1

Geographical coordinates of sites on the Verdon River and in Lake Sainte-Croix

Sites (numbers)	Geographical coordinates	
Verdon at the town of Brans (1)	N43° 49′ 41.4123″	E6° 27′ 52.3416″
Verdon at the Bridge of the Estellier (2)	N43° 44′ 34.3551″	E6° 20′ 30.9486″
Verdon at the point where it flows into Lake Sainte-Croix near the Pont du Galetas bridge (3)	N43° 48′ 5.1486″	E6° 14′ 57.9342″
Littoral zone of Lake Sainte-Croix – the town of Les Salles sur Verdon (4)	N43° 46′ 42.0607″	E6° 12′ 27.9193″
Littoral zone of Lake Sainte-Croix – the town of Bauden (5)	N43° 44′ 13.7611″	E6° 7′ 57.9002″
Littoral zone of Lake Sainte-Croix – the town of St Crox-de-Verdon (6)	N43° 45′ 24.1296″	E6° 9′ 4.1401″
Verdon outflow from Lake Sainte-Croix (7)	N43° 44′ 13.7890″	E6° 7′ 56.4325″

reasons, but they are planned to be sampled in the next series of research. At each test stand, 1 dm^3 samples of water were drawn into PET bottles. The water pH (PN-EN ISO 10523:2012) was determined on site with a pH meter PH-207 Slandi, while electrolytic conductivity (PN-EN 27888:1999) was measured as the same time with a conductivity meter CM 204 Slandi. The collected water samples were transported to the laboratory under cool conditions, and there the content of metals (Cu, Cd, Pb, Zn, Ni) was determined (PB-10/I - 1998).

In addition, only submerged aquatic plants were collected for testing, which was a purposeful choice because the objective was to test pollutants in plants whose whole surface was in contact with water (PN-EN ISO 5667-6:2016-12; PN-ISO 5667-4:2017-10). The flora was dominated by the genus *Potamogeton* (*P. pectinatus, P. nodosus, P. lucens*). The samples also included *Ceratophyllum demersum* and *Myriophyllum alterniflorum*. Whole plants were taken for testing. Only one plant species was collected at every site, and the number of samples from each site was *N*=5. In total, 35 plants were collected.

The plants were washed several times with water from the lake to remove impurities, then rinsed three times in redistilled water, and maintained at room temp. (20-22°C) until dry (natural method) (Król, Kiełtyka-Dadasiewicz 2015). The samples were cut, ground and homogenised. 0.5 g of each sample was transferred to an HP-500 Teflon vessel and mineralized in a Mars 5 CEM microwave oven with 5 cm^3 concentrated HNO_3 using a three-step mineralization procedure. After cooling to room temperature, the supernatants were transferred to serological tubes and diluted with redistilled water to 25 cm^3. Metal concentrations (Cu, Cd, Pb, Zn, Ni) were determined using atomic absorption spectroscopy on a Varian SpectrAA-110/220 unit (PB-10/I – 1998). The detection limit was 0.10 µg dm^{-3}. The results are given in mg kg^{-1} of dry mass for plants, and in µg dm^{-3} for water.

The accuracy of the analytical procedures was verified using Certified Reference Material BCR No 60 (*Lagarosiphon major*), The Commission of the European Communities, The Community Bureau of Reference – BCR (Table 2).

Table 2

Reference values of metals and values determined in the study (mg kg^{-1})

Metal	Reference values	Values obtained
Cu	51.0 ± 1.90	48.86 ± 0.67
Cd	2.20 ± 0.10	2.240 ± 0.05
Pb	63.80 ± 3.20	59.72 ± 0.49
Zn	313.0 ± 8.00	311.1 ± 7.54
Ni	40.00*	37.53 ± 2.07

* Values given by the manufacturer, but not reference values.

Statistical analysis

The bioaccumulation factor (BCF) for metals in plants was computed by dividing the metal concentration in the plant by its concentration in water, following the method given by Nguyen et al. (2005):

$$BCF = C_P/C_W,$$

where: BCF – bioaccumulation factor, C_P – metal concentration in the plant, C_W – metal concentration in the water.

In addition, metal pollution indices (MPIs) were calculated for water and for aquatic plants at the study sites, according to the formula (Usero et al. 1996):

$$MPI = (Cf_1 \cdot Cf_2 \ldots Cf_n)^{1/n},$$

where: C_f – metal concentration in a sample.

Statistical analysis of the results was performed with Statistica 10.0. Correlations between respective parameters were calculated using the Spearman's coefficient. Normality of distribution was verified with the Shapiro-Wilk test. Subsequently, the Kruskal-Wallis test was used, because normality of distribution and *post-hoc* analysis were not confirmed (ZAR 1999). The PCA was calculated using *r*-statistic.

RESULTS AND DISCUSSION

Water pH was found to oscillate around neutral (pH of 6.50-7.64) – Table 3. The Verdon River water flowing from Lake Sainte-Croix had a lower pH (mean pH 6.80) than the water flowing into the lake (mean pH 7.41). The mean lake water pH was 6.97. The pH of the water in the reservoir varied between the sites, which is indicated by a gradual decrease of the pH value, starting from the uppermost part of the reservoir to the areas adjacent to the dam. The water is considered to be of class 1 quality according to the ranges for surface water in Europe, pH 6.50-9.00 (Directive 2000/60/EC).

Table 3

Water reaction (pH), electrolytic conductivity (μS cm^{-1}) and metal (μg dm^{-3}) concentrations in the water of the Verdon River and Lake Sainte-Croix

Sites (number)	Water reaction	Electrolytic conductivity	Cu	Ni	Cd	Pb	Zn
	min.-max. $\bar{x} \pm SD$						
1	7.23 - 7.44 7.36 ± 0.10	409 - 420 414.0 ± 4.55	5.60 - 6.00 5.80 ± 0.16	0.10 - 0.30 0.20 ± 0.08	0.30 - 0.50 0.40 ± 0.08	0.10 - 0.20 0.13 ± 0.05	9.80 - 20.0 13.27 ± 4.76
2	7.58 - 7.64 7.61 ± 0.02	382 - 390 385.7 ± 3.30	3.00 - 3.20 3.10 ± 0.08	0.10 - 0.30 0.17 ± 0.09	0.20 - 0.40 0.30 ± 0.08	0.10 - 0.30 0.20 ± 0.08	16.80 - 18.00 17.53 ± 0.52
3	7.39 - 7.43 7.41 ± 0.02	468 - 476 471.7 ± 3.30	4.50 - 4.70 4.60 ± 0.08	1.40 - 1.60 1.50 ± 0.08	0.10 - 0.30 0.20 ± 0.08	0.10 - 0.20 0.13 ± 0.05	16.10 - 18.00 17.00 ± 0.78
4	7.56 - 7.62 7.59 ± 0.02	368-374 371.0 ± 2.45	2.30 - 2.50 2.40 ± 0.08	1.11 - 1.20 1.07 ± 0.09	0.50 - 0.70 0.60 ± 0.08	0.10 - 0.30 0.17 ± 0.09	9.80 - 10.60 10.13 ± 0.34
5	6.70 - 6.85 6.78 ± 0.06	370 - 376 373.7 ± 2.62	3.20 - 3.40 3.30 ± 0.08	0.80 - 1.00 0.90 ± 0.08	0.30 - 0.50 0.40 ± 0.08	0.10 - 0.20 0.13 ± 0.05	12.50 - 14.00 13.40 ± 0.65
6	6.50 - 6.56 6.53 ± 0.02	374 - 378 376.0 ± 1.63	2.40 - 2.70 2.53 ± 0.12	0.50 - 0.70 0.60 ± 0.08	0.30 - 0.50 0.40 ± 0.08	0.10 - 0.30 0.20 ± 0.08	9.00 - 11.00 10.17 ± 0.85
7	6.75 - 6.85 6.80 ± 0.04	401 - 406 404.0 ± 2.16	5.30 - 5.50 5.40 ± 0.08	0.80 - 1.00 0.90 ± 0.08	0.40 - 0.60 0.50 ± 0.08	0.10 - 0.30 0.20 ± 0.08	17.00 - 19.00 18.17 ± 0.85
Mean value ($\bar{x} \pm SD$)	7.16 ± 0.41	399.43 ± 33.22	3.88 ± 1.28	0.76 ± 0.45	0.40 ± 0.14	0.17 ± 0.08	14.24 ± 3.67

Values are not very low, and there is a tendency of decreasing pH values in the reservoir and in the water it discharges. However, the pH in the reservoir should be monitored, as it is rather acidic.

Compared to this, the water of the Verdon River tested for over a year downstream off the reservoir by BERTRAND et al. (2001) had higher pH values (between pH 8.10 and pH 8.20), or slightly more alkaline than upstream and in the reservoir. It can therefore be assumed that the varying pH values are a result of fluctuations in the reservoir, and should be further investigated or at least monitored.

The maximum value of water mineralization expressed as electrolytic conductivity was 476 μS cm^{-1}, thus being typical of slightly polluted water (Table 3). The water in the river upstream was more mineralized (mean 471.67 μS cm^{-1}) than in the lake (mean 373.00 μS cm^{-1}) or downstream (mean 404.00 μS cm^{-1}). An increase in conductivity values was observed in the reservoir along with the direction of water flow, which may indicate gradual accumulation of mineral compounds and their displacement towards the outflow. This manifests sedimentation and accumulation of mineral compounds brought by the river from the catchment area. Mineral compounds can be deposited in the reservoir, accumulated in sediment and incorporated in living organisms, which results in lower conductivity values of lake water. Therefore, the role of the lake and its accumulation can be considered as a positive one. Similar values were noted by BERTRAND et al. (2001) for the Verdon River in an annual cycle of tests carried out downstream from the reservoir (390 - 401 μS cm^{-1}), which proves that this is a long-term tendency. Correlations between all the parameters tested were determined (Table 4). The most statistically significant correlations were found between the plant species and the accumulation of metals tested. Zinc was an exception (Figure 2). This is reflected in PCA (Figure 3), even though it was impossible to draw an ellipse for certain species due to their small number. No statistically significant correlations were found between metal content in water and in plants.

Heavy metals show different patterns of behaviour in water than most chemicals. They are not or biologically formed or degraded, but only undergo chemical conversion. As they occur naturally, they may be absorbed by and accumulated in living organisms. Some of them (Cr, Co, Cu, Fe, Mn, Mo, Ni, Se, Zn) are important for the function of organisms when present at adequate levels, but others are undesirable (As, Sb, Cd, Pb, Hg, Tl, Ag, Sn) and should not be incorporated into living structures. Therefore, both their deficiency and excess may result in various deformations. Toxic effects of metals on aquatic living organisms are closely related to conditions in water bodies. When conditions are suitable, even high metal levels may show no effects because their negative impact is somehow suppressed. However, with changed environmental conditions related to pH, water hardness, organic carbon content and sulphur content, unfavourable effects of heavy metals

Table 4

Spearman correlation

Spearman correlation	pH	Conductivity	Cu_w	Ni_w	Cd_w	Pb_w	Zn_w	Cu_p	Ni_p	Cd_p	Pb_p	Zn_p	Site
Conductivity	0.05												
Cu_w	-0.11	0.75*											
Ni_w	-0.12	0.02	-0.13										
Cd_w	0.04	-0.52*	-0.23	0.00									
Pb_w	-0.06	-0.16	-0.09	-0.14	0.20								
Zn_w	0.12	0.49*	0.47*	-0.07	-0.14	0.28							
Cu_p	0.20	-0.62*	-0.41	0.46*	0.42	0.05	0.04						
Ni_p	0.17	-0.85*	-0.83*	-0.26	0.44*	0.22	-0.53*	0.32					
Cd_p	0.38	0.14	0.09	0.22	0.06	0.05	0.60*	0.52*	-0.25				
Pb_p	0.24	0.47*	0.34	0.45*	-0.29	0.00	0.72*	0.29	-0.66*	0.75*			
Zn_p	0.55*	0.28	-0.18	0.46*	-0.15	0.09	0.27	0.18	-0.09	0.52*	0.55*		
Site	-0.70*	-0.38	-0.23	0.35	0.37	0.18	0.01	0.39	0.07	0.18	0.04	-0.20	
Plant	-0.13	0.07	0.25	0.54*	0.05	-0.01	0.51*	0.61*	-0.49*	0.68*	0.77*	0.11	0.49*

M_w – content of metals in water, M_p – content of metals in aquatic plants, * $P<0.05$

may become apparent and the elements can be absorbed by living organisms (Mohiudin et al. 2012).

The mean concentrations of metals in the all water samples were as follows: Cu (3.88 μg dm^{-3}), Ni (0.76 μg dm^{-3}), Cd (0.40 μg dm^{-3}), Pb (0.17 μg dm^{-3}) and Zn (14.24 μg dm^{-3}) – Table 3. At their maximum levels, all the metals tested fell within the ranges established for class 1 quality for surface water bodies monitored in Europe (Directive 2000/60/EC). The metal levels in the tested water samples can be ordered as follows: Pb<Cd<Ni<Cu<Zn.

The level of Ni in the water entering the reservoir was slightly higher than downstream off the lake (Table 3). This implicates small but noticeable accumulation in the reservoir. Ni may be incorporated both in sediment structures and in living tissues. Contrary findings were achieved for the other metals: their higher levels appeared in the water leaving the reservoir. This may be an effect of water being discharged from the reservoir because the dam has a bottom outlet. Bottom sediment can be the source of metals mobilized with water flow. However, no samples of bottom sediments were collected in our study and the above can be treated merely as a presumption.

The horizontal profile of the reservoir shows that the concentrations of copper, lead and zinc decreases with the water flow direction, probably

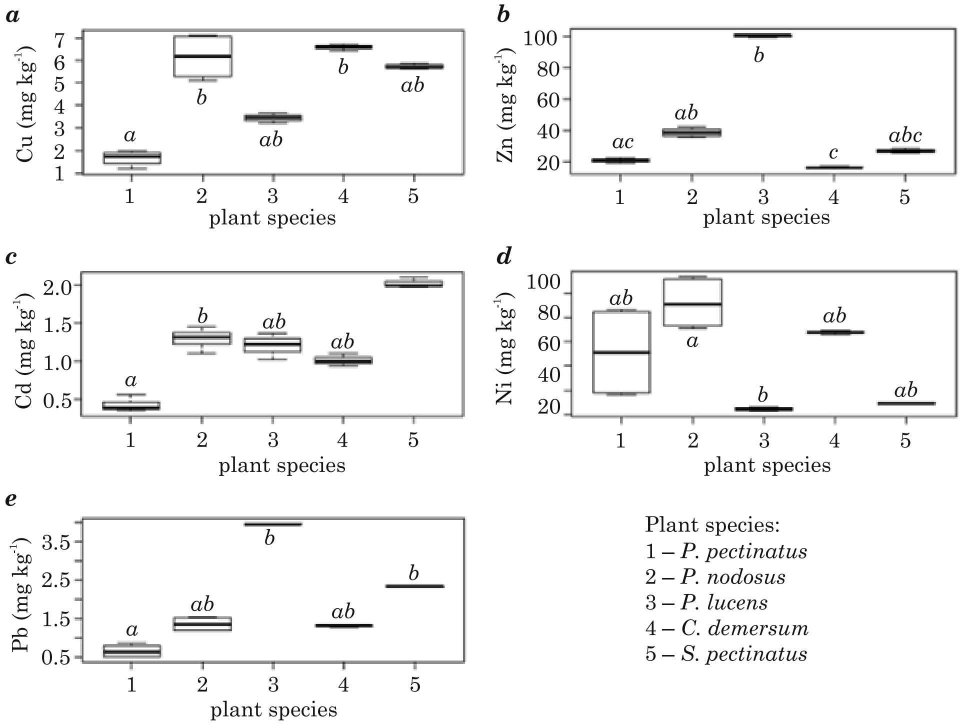


Fig. 2. Content of metals in aquatic plants depending on the species.
Values marked with the same letter are not significantly different from one another $P > 0.05$

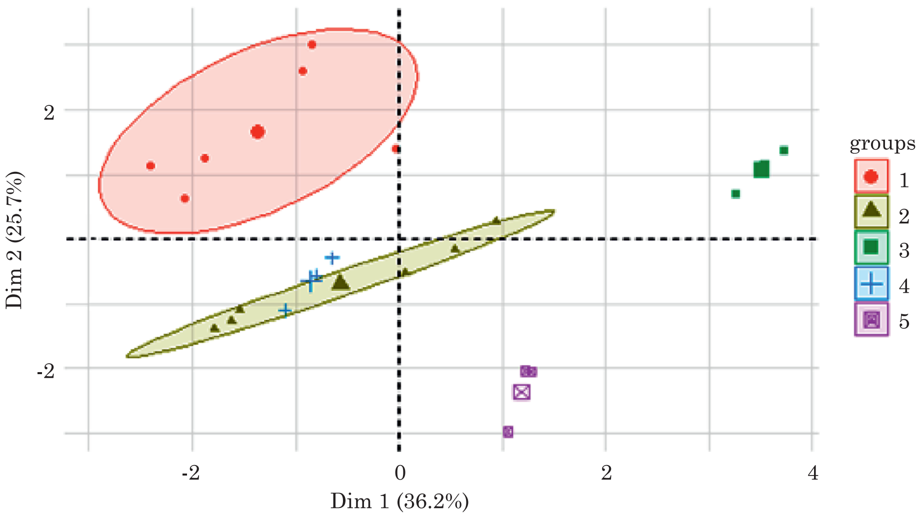


Fig. 3. PCA based on concentrations of elements in whole plant of water plants.
Numbers refer to plant species: 1 – *S. pectinatus*, 2 – *P. nodosus*, 3 – *P. lucens*, 4 – *C. demersum*, 5 – *S. pectinatus*

as a result of their accumulation in the sediment or incorporation into living organisms. The nickel and cadmium levels in water increasing along the horizontal profile may be due to conditions conducive to their release from the bottom sediment or living organisms, or else they implicate the effect of dry deposition from the atmosphere directly above the reservoir. There were no differences in the levels of lead ions between the sites and no clear relationships between the variables were found.

The assessment of the level of metals in water was carried out on the basis of a comparison with literature data. Similar cadmium, copper and zinc levels as those found in the Verdon and Lake Sainte-Croix are also seen in mountainous flowing or standing water (dam reservoirs) of the temperate zone, but without a large pollution load (MATACHE et al. 2013, POKORNY et al. 2015). Copper and zinc levels of the Verdon were very similar to those found in rivers Parsęta and Radew (Poland), which do not have large pollution loads even though they are lowland rivers (SENZE et al. 2018). Similar findings were reported in a study on the Narew River by SKORBIŁOWICZ (2009, 2015).

Higher levels of heavy metals than in the Verdon River and Lake Sainte-Croix are typically detected in rivers and reservoirs with standing water which accumulate anthropogenic pollution (household and industrial) both from urban and agricultural areas. The water of the Odra River has a higher metal content than the Verdon (KLINK et al. 2016). Similarly, metal levels were higher in lake water near the city of Poznań (SZYMANOWSKA et al. 1999). Higher concentrations of heavy metals were also found in water from small rivers in south-western Poland (KLINK et al. 2013), rivers in Japan (MOHIUDDIN et al. 2012), and the Danube in Romania (MATACHE et al. 2013). Similar levels were also noted in China in rivers that received municipal sewage, and in the Euphrates in Egypt, which drained an urbanized area (PENG et al. 2008, SALAH et al. 2015).

The mean concentrations of heavy metals in the studied hydromacrophytes were as follows: Cu (4.49 mg kg^{-1}), Ni (5.26 mg kg^{-1}), Cd (1.10 mg kg^{-1}), Pb (1.67 mg kg^{-1}), Zn (37.64 mg kg^{-1}) – Table 5. Lead and zinc content in plants collected upstream from the reservoir was higher than in the reservoir and downstream. In addition, comparisons of metal concentrations at the sampling sites were made. Differences were seen, but results for each metal were varied (Figure 4). Statistically significant differences were found for copper between sites 1 and 4, for zinc between sites 3 and 5, for nickel between sites 3 and 4, for cadmium between sites 1 and 7 and 6 and 7 and for lead between sites 3 and 6.

Cadmium, copper and nickel levels were the highest in plants at the outlet. The content of metals in the tested aquatic plants can be ordered as follows: Cd<Cu<Ni<Pb<Zn. In parallel with the overall evaluation, an analysis of metal content in aquatic plants entailed comparison of two species: *Potamogeton pectinatus* and *Potamogeton nodosus*, whose specimens

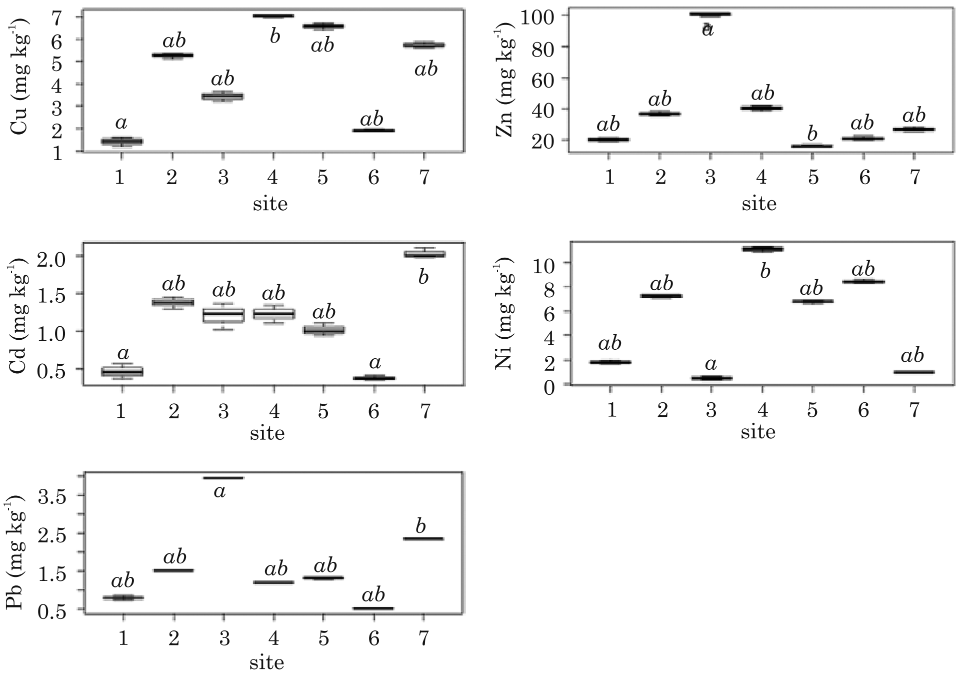


Fig. 4. The content of metals in aquatic plants depending on the site.
Values marked with the same letter are not significantly different from one another $P > 0.05$

Table 5

Metal concentrations (mg kg^{-1}) in aquatic plants of the Verdon River and Lake Sainte-Croix

Sites (number) and plant species	Cu	Ni	Cd	Pb	Zn
	min. - max. $\bar{x}$ ± SD				
1 *Potamogeton pectinatus*	1.20 - 1.61 1.42 ± 0.17	1.655 - 1.97 1.79 ± 0.13	0.37 - 0.57 0.47 ± 0.08	0.74 - 0.85 0.799 ± 0.05	19.02 - 21.52 20.48 ± 1.06
2 *Potamogeton nodosus*	5.13 - 5.40 5.28 ± 0.11	7.10 - 7.31 7.23 ± 0.09	1.29 - 1.45 1.38 ± 0.06	1.52 - 1.54 1.54 ± 0.01	35.65 - 38.52 37.03 ± 1.18
3 *Potamogeton lucens*	3.22 - 3.65 3.44 ± 0.18	0.32 - 0.64 0.47 ± 0.13	1.03 - 1.37 1.21 ± 0.14	3.95 - 3.97 3.96 ± 0.01	99.57 - 101.62 100.7 ± 0.86
4 *Potamogeton nodosus*	6.97 - 7.10 7.05 ± 0.06	10.89 - 11.36 11.15 ± 0.19	1.10 - 1.34 1.22 ± 0.09	1.19 - 1.21 1.20 ± 0.01	38.65 - 42.25 40.59 ± 1.48
5 *Ceratophyllum demersum*	6.42 - 6.75 6.59 ± 0.13	6.63 - 6.95 6.78 ± 0.13	0.95 - 1.10 1.02 ± 0.06	1.29 - 1.33 1.31 ± 0.01	15.85 - 16.97 16.35 ± 0.47
6 *Potamogeton pectinatus*	1.87 - 1.99 1.92 ± 0.05	8.33 - 8.64 8.48 ± 0.13	0.35 - 0.42 0.38 ± 0.03	0.52 - 0.54 0.53 ± 0.01	20.15 - 22.53 21.34 ± 0.97
7 *Myriophyllum alterniflorum*	5.63 - 5.87 5.74 ± 0.09	0.89 - 1.00 0.94 ± 0.043	1.99 - 2.10 2.03 ± 0.05	2.32 - 2.35 2.34 ± 0.01	25.64 - 28.52 26.96 ± 1.19
Mean value ($\bar{x}$ ± SD)	4.49 ± 2.08	5.26 ± 3.87	1.10 ± 0.53	1.67 ± 1.07	37.64 ± 27.05

were collected from the Verdon River upstream off the reservoir and in it. Therefore, metal accumulation in plant tissues could be evaluated. The trend of changes was identical for both species. Copper, nickel and zinc content was higher in plants collected from the reservoir than in those from the Verdon. Conversely, cadmium and lead were higher in plants from the reservoir. Lead, nickel, zinc and cadmium levels in aquatic plants harvested from the Verdon River and Sainte-Croix Lake were similar to the ones detrmined in hydromacrophytes from rivers free from pollution load, both in mountainous areas, such as Sainte-Croix Lake, and in lowland areas (Pokorny et al. 2015, Senze et al. 2018).

Much higher values of the tested metals were accumulated in plants found in flowing and standing water in urbanized areas. Geographic location was of no relevance here because such relationships were found both in Europe and in the other continents, regardless of what type of water bodies, flowing or standing, were studied (Szymanowska et al. 1999, Peng et al. 2008, Rai 2009, Jastrzębska et al. 2010, Rajfur et al. 2010, Klink et al. 2013, Matache et al. 2013, Philips et al. 2015, Skorbiłowicz 2015, Gao et al. 2016, Klink et al. 2016, Parzych et al. 2016).

Metal pollution indices (MPIs) for water are ordered as follows for the sites: 2 < 1 < 6 < 5 < 3 < 4 < 7; MPIs for aquatic plants: 1 < 6 < 7 < 3 < 5 < < 2 < 4 (Table 6). These calculations and their analysis show that pollution

Table 6

Metal pollution index (MPI) – water and aquatic plants

Sites (number)	MPI	
	water	plants
1	0.0009	1.797
2	0.0008	4.948
3	0.0012	3.754
4	0.0012	5.419
5	0.0011	3.956
6	0.0010	2.336
7	0.0015	3.698

levels in water are typical of dam reservoirs. The results implicate the role of dam reservoirs in water retention. Water entering a reservoir has a lower metal content than that in it. The highest levels are found in discharged water. For plant samples, in turn, the lowest MPI was found at site 1. This may be accounted for by a higher water flow in this part of the river, possibly low metal content in bottom sediment and also easily accumulated pollutants that plants could capture from the sediment. The highest values found at site 7 (downstream off the reservoir) are a result of pollution leaking from the reservoir with water and accumulation (most likely in the sedi-

Table 7

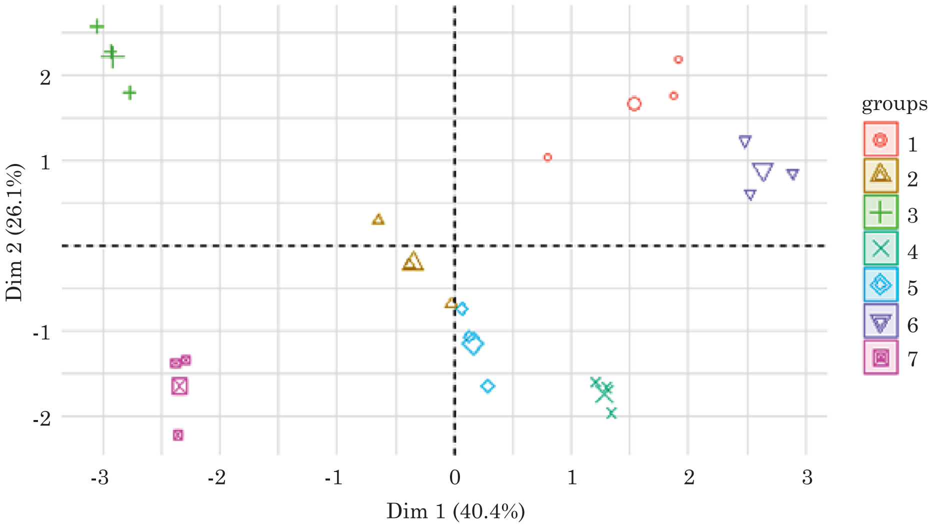
Bioconcentration factor (BCF) of metals in aquatic plants at sites

Sites (number)	Cu	Ni	Cd	Pb	Zn
	min. - max. $\bar{x}$ ± SD				
1	200 - 288 244 ± 36	5515 - 19 652 11 315 ± 6043	1129 - 1217 1168 ± 36	4263 - 8067 6577 ± 1659	1076 - 2089 1702 ± 446
2	1687 - 1709 1701 ± 10	24 367 - 72 829 56 070 ± 22 429	3236 - 7251 5033 ± 1665	5075 - 15391 9365 ± 4386	2073 - 2140 2112 ± 28
3	683 - 811 749 ± 52	225 - 399 311 ± 71	3418 - 13658 7736 ± 4331	19 839 - 39 578 32 977 ± 9289	5645 - 6184 5934 ± 221
4	2841 - 3028 2939 ± 76	9467 - 11186 10 515 ± 750	1915 - 2201 2054 ± 116	3980 - 12 103 9372 ± 3813	3857 - 4225 4009 ± 157
5	1983 - 2005 1996 ± 9	6632 - 8682 7613 ± 839	1891 - 3668 2688 ± 736	6626 - 13 132 10 885 ± 3012	1132 - 1357 1224 ± 96
6	737 - 779 759 ± 17	11 900 - 17 274 14 429 ± 2205	703 - 1239 993 ± 220	1775 - 5266 3228 ± 1484	2031 - 2238 2106 ± 94
7	1024 - 1107 1062 ± 34	895 - 1251 1062 ± 146	3507 - 4964 4158 ± 604	7841 - 23 392 14 286 ± 6621	1444 - 1507 1484 ± 28
Mean value ($\bar{x}$ ± SD)	1350 ± 853	14 474 ± 19 745	3405 ± 2869	12 385 ± 10 307	2653 ± 1594

ment). These are probably the sources of heavy metals for plants. The situation varies within the reservoir, depending on the type of shore and its development.

Mean metal accumulation expressed as BCFs was: Cu (BCF = 1350), Ni (BCF = 14474), Cd (BCF = 3405), Pb (BCF = 12385), Zn (BCF = 2653) – Table 7. The highest BCF values for the heavy metals in question were found in the lake. As for the reservoir's horizontal profile (with the water flow direction), a decrease in BCF for the subsequent sites appears for all metals. The analysis did not confirm statistically significant correlations between metal levels in water and in plants. However, ellipses could not be drawn due to an insufficiently high number of tests. Differences in terms of sites can be seen based on all metals (Figure 5).

In the inflow/outflow analysis, higher values were noted for cadmium, lead and zinc in the inflow than in the outflow. Nickel and copper concentrations were higher downstream than upstream. The bioaccumulation of nickel, cadmium and lead in plants of Lake Sainte-Croix and the Verdon River was similar as in mountainous and lowland rivers (Pokorny et al. 2015, Senze et al. 2018). An analysis of the bottom sediment composition, which has not been performed in this study, would certainly give a clearer picture of possible bioaccumulation of metals.



Groups: 1 - 7 sites

Fig. 5. PCA based on concentrations of elements in leaves of water plants relative to the location of study sites

CONCLUSIONS

Metal concentrations in the samples were similar to those found in moderately polluted surface water. A general tendency for metal accumulation in the reservoir has been demonstrated. A positive role of the reservoir can be identified as a site of potential accumulation of heavy metals. Differences between the sites can be seen based for all metals. Correlations between the tested parameters were determined, and the most statistically significant correlations were found between the plant species and accumulation of all the metals tested (except zinc), which was also confirmed by PCA. No statistically significant correlations were found between metal content in water and in plants. It is possible that an analyses of the metal content in bottom sediments, in the immediate vicinity of the reservoir, e.g. in the soil and in other components of the catchment area, which is planned in our further research, will resolve the above question.

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