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USING BIOINDICATORS TO ASSESS HEAVY METAL CONTAMINATION IN SURFACE WATERS AND PUBLIC HEALTH IMPACTS

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ABSTRACT

Objective: This study aims to explore the use of bioindicators for assessing metal contamination in surface waters and its effects on public health.

Theoretical Framework: The persistence and variation of heavy metal contamination in the Ave River Basin over 15 years underscore the need for ongoing monitoring and intervention. The detection of elevated levels of chromium, cadmium, lead, and zinc in aquatic mosses highlights the continuing impact of industrial activities, particularly metal coating facilities, on the river's ecosystem.

Method: In the study area, the Ave River and some of its tributaries, a monitoring programme of twelve sampling points was defined in two campaigns and eight metallic ions were determined in the laboratory by atomic absorption spectrophotometry (Cd, Pb, Cu, Cr, Fe, Hg, Ni and Zn).

Results and Discussion: The highest metal accumulation in mosses was observed for iron (Fe), whereas the lowest accumulation was found for mercury (Hg) during Campaign II. The order of metal accumulation in the moss samples, from highest to lowest, was Fe > Zn > Cu > Cr > Ni > Pb > Cd > Hg. The Metal Pollution Index (MPI) revealed changes in contamination levels between campaigns.

Research Implications: The contamination patterns suggest the influence of industrial activities, particularly metal coating facilities. Monitoring and mitigation efforts are necessary to address persistent heavy metal pollution in the Ave River Basin.

Originality/Value: The results of this research will contribute to a better understanding of the sources and loads of pollutants discharged and responsible for the contaminants. This can be used to discourage potential polluters and better manage the basin's water resources and possible risks to human health.

Keywords: Aquatic Moss, Ave River Basin, Biomonitoring, Metal Pollution Index, Public Health, Water Pollution.

UTILIZAÇÃO DE BIOINDICADORES PARA AVALIAR A CONTAMINAÇÃO POR METAIS PESADOS EM ÁGUAS DE SUPERFÍCIE E OS IMPACTOS NA SAÚDE PÚBLICA

RESUMO

Objetivo: Este estudo tem como objetivo explorar a utilização de bioindicadores para avaliar a contaminação por metais em águas superficiais e seus efeitos na saúde pública.

Estrutura teórica: A persistência e variação da contaminação por metais pesados na Bacia do Rio Ave ao longo de 15 anos reforçam a necessidade de monitoramento e intervenção contínuos. A detecção de níveis elevados de crômio, cádmio, chumbo e zinco em musgos aquáticos destaca o impacto contínuo das atividades industriais, particularmente instalações de revestimento de metais, no ecossistema do rio.

Método: Na área de estudo, o rio Ave e alguns de seus afluentes, foi definido um programa de monitoramento de 12 pontos de amostragem em duas campanhas e oito íons metálicos foram determinados em laboratório por espectrofotometria de absorção atômica (Cd, Pb, Cu, Cr, Fe, Hg, Ni e Zn).

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Resultados e Discussão: A maior acumulação de metais em musgos foi observada para o ferro (Fe), enquanto a menor acumulação foi encontrada para o mercúrio (Hg) durante a Campanha II. A ordem de acumulação de metais nas amostras de musgo, do mais alto ao mais baixo, foi Fe > Zn > Cu > Cr > Ni > Pb > Cd > Hg. O Índice de Poluição Metálica (IPM) revelou mudanças nos níveis de contaminação entre as campanhas**.**

Implicações da pesquisa: Os padrões de contaminação sugerem a influência de atividades industriais, particularmente instalações de revestimento de metais. São necessários esforços de monitorização e mitigação para fazer face à poluição persistente por metais pesados na bacia do rio Ave.

Originalidade/valor: Os resultados desta pesquisa contribuirão para uma melhor compreensão das fontes e cargas de poluentes descarregados e responsáveis pelos contaminantes. Isso pode ser usado para desencorajar potenciais poluidores e gerenciar melhor os recursos hídricos da bacia e possíveis riscos para a saúde humana.

Palavras-chave: Musgo Aquático, Bacia Hidrográfica Ave, Biomonitorização, Índice de Poluição Metálica, Saúde Pública, Poluição da Água.

UTILIZACIÓN DE BIOINDICADORES PARA EVALUAR LA CONTAMINACIÓN POR METALES PESADOS EN LAS AGUAS SUPERFICIALES Y LOS EFECTOS EN LA SALUD PÚBLICA

RESUMEN

Objetivo: Este estudio tiene como objetivo explorar el uso de bioindicadores para evaluar la contaminación de metales en aguas superficiales y sus efectos en la salud pública.

Marco teórico: La persistencia y variación de la contaminación por metales pesados en la cuenca del río Ave a lo largo de 15 años pone de relieve la necesidad de una vigilancia e intervención continuas. La detección de niveles elevados de cromo, cadmio, plomo y zinc en los musgos acuáticos pone de relieve el impacto continuo de las actividades industriales, en particular las instalaciones de recubrimiento de metales, en el ecosistema del río.

Método: En el área de estudio, el río Ave y algunos de sus afluentes, se definió un programa de monitoreo de doce puntos de muestreo en dos campañas y se determinaron ocho iones metálicos en el laboratorio por espectrofotometría de absorción atómica (Cd, Pb, Cu, Cr, Fe, Hg, Ni y Zn).

Resultados y Discusión: La mayor acumulación de metales en los musgos se observó para el hierro (Fe), mientras que la menor acumulación se encontró para el mercurio (Hg) durante la Campaña II. El orden de acumulación de metales en las muestras de musgo, de mayor a menor, fue Fe > Zn > Cu > Cr > Ni > Pb > Cd > Hg. El Índice de Contaminación Metálica (IPM) reveló cambios en los niveles de contaminación entre campañas.

Implicaciones de la investigación: Los patrones de contaminación sugieren la influencia de las actividades industriales, particularmente las instalaciones de recubrimiento de metales. Es necesario realizar actividades de vigilancia y mitigación para hacer frente a la contaminación persistente por metales pesados en la cuenca del río Ave.

Originalidad/Valor: Los resultados de esta investigación contribuirán a una mejor comprensión de las fuentes y cargas de contaminantes descargados y responsables de los contaminantes. Esto puede utilizarse para desalentar a los contaminadores potenciales y gestionar mejor los recursos hídricos de la cuenca y los posibles riesgos para la salud humana.

Palabras clave: Musgo Acuático, Cuenca Del Río Ave, Biomonitoreo, Índice De Contaminación Metálica, Salud Pública, Contaminación Del Agua.

1 INTRODUCTION

The current situation presents a realistic scenario in which humans' utilisation of metals has significantly impacted the environment since the onset of the Industrial Revolution. Two centuries after the Industrial Revolution, we are now situated in what can be called the "Metal Removal Age". This is when we collectively understand the hazards linked to the unregulated spread of heavy metals in the environment. The distribution of metals occurs across various terrestrial compartments, including water, air, and soil, as a result of natural phenomena such as leaching and weathering of igneous or metamorphic rocks, as well as human activities such as the discharge of urban and industrial wastewater (Tutic *et al.*, 2015; Wu *et al.*, 2016).

The presence of heavy metals in surface water has been confirmed. By monitoring surface and groundwater quality and implementing control measures and sanitary surveillance for human consumption, it has become evident that various metallic ions exist in concentrations exhibiting significant temporal and spatial variations. Metallic ions are recognised as substantial pollutants in the aquatic environment because of their prolonged persistence in the ecosystem, toxicity even at low concentrations, and ability to readily enter and accumulate within food chains. This attribute constitutes a significant concern for ecosystem integrity and, ultimately, human well-being, given that humans occupy the uppermost trophic levels in various food chains (Abdel *et al.*, 2011; Tesser *et al.*, 2021).

Aquatic mosses, particularly bryophytes, have emerged as highly suitable bioindicators in marine ecosystems. According to Zechmeister *et al.* (2003), bryophytes fulfil all the essential criteria for an effective indicator: ease of collection, tolerance to high metal concentrations, convenient laboratory handling, accumulation of sufficient metal quantities for analysis without pre-concentration, and establishment of a direct correlation between the accumulated metal concentration and the surrounding environment (Martins, 2004; Vuori and Helisten, 2010). Additionally, their capacity to accumulate metals in aqueous solutions is notably high due to the absence of a cuticle in their tissues and the abundance of cation exchange sites on their cell walls (Macedo-Miranda *et al.*, 2016). Numerous moss species have been successfully used as bioindicators of heavy metal contamination in aquatic ecosystems (; Klos *et al.*, 2011; Varela *et al.*, 2015; Barbosa and Carvalho, 2016; Macedo-Miranda *et al.*, 2016).

In a study conducted by Gonçalves *et al.* (1994), the concentrations of a specific set of metals were assessed in aquatic moss samples collected from multiple sampling stations within the Rio Ave Basin. The collected data facilitated the classification and ranking of the sampled sites by utilising parameters such as the Contamination Factor and Metal Pollution Index.

2 OBJECTIVES

This study aims to explore the use of bioindicators for assessing metal contamination in surface waters and its effects on public health. These findings provide critical insights for shaping policy and allocating resources to effectively manage heavy metal contamination in domestic drinking water.

3 METHODOLOGY

3.1 STUDY AREA

The Ave River has a length of approximately 98 km, and its drainage basin (Fig. 1), located in Northern Portugal, covers an area of approximately 1388 km^2. The most significant tributaries are the Vizela River (47 km long, with a drainage area of 342 km^2) on the left riverbank and the Este River (52 km, 246 km^2) on the right riverbank.

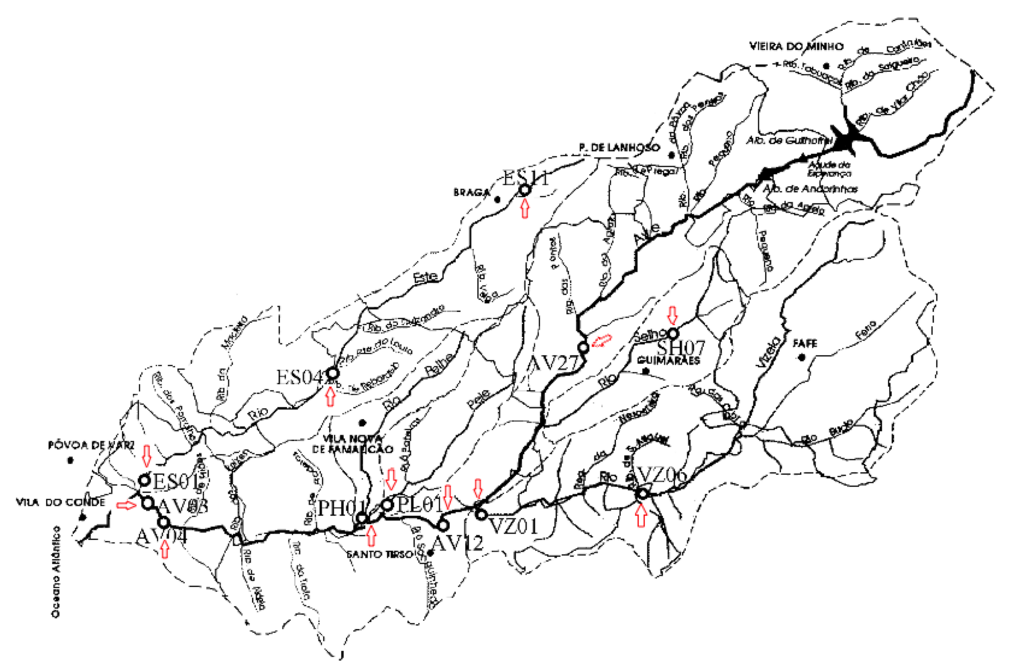
The Pelhe and Pele Rivers flow almost side by side, with a length of 20 km and drainage areas of 44 km^2 and 61 km^2, respectively. The Selho River, covering an area of 59 km^2 and 20 km in length, is also present in this area.

The Ave River Basin is located in one of the rainiest regions of the country, with an average annual precipitation of 1800 mm.

Due to this region's high level of industrialisation, some water quality issues have been observed. The textile industry is the most prominent sector, accounting for approximately 70% of the workforce. Other significant industries include tanneries, rubber manufacturing, plastic production, and surface coating (chroming and nickel plating). A considerable percentage of industrial effluents is discharged directly into rivers without proper treatment.

Figure 1

Drainage basin of River Ave, sampling stations.



3.2 SAMPLING

The Ave For comprehensive environmental monitoring, a meticulous selection process was undertaken to establish optimal sampling stations (ST) along various sections of the Ave River (AV) – 4 ST, Este River (ES) – 3ST, and Vizela River (VZ) – 2 ST, as well as a few smaller tributaries including the Pele River (PL), Pelhe River (PH), and Selho River (SH), one ST in each. Several crucial factors were considered in the selection process.

Figure 1 shows the sampling stations located in the study area. The corresponding geographical coordinates of each station are listed in Table 1. These coordinates are crucial for accurately identifying and georeferencing each sampling point in the river basin. Furthermore, geographical coordinates also facilitate the comparison and sharing data with other studies and research conducted in the same region or adjacent areas.

First, historical data played a vital role in identifying critical locations for monitoring. Contamination and pollutant dispersion patterns were identified by analysing previous records, aiding in determining appropriate sampling points. Additionally, bryophytes, sensitive water quality indicators, were observed at specific sites, further guiding the selection process.

Table 1

Sampling points in the study area (GPS coordinates).

Sampling point	N	W
ES01	41°22'22.97"	8°42'9.03"
ES04	41°26'38.30"	8°32'53.34"
ES11	41°33'48.56"	8°22'22.49"
AV03	41°22'0.14"	8°41'47.42"
AV04	41°21'4.48"	8°40'54.20"
PH01	41°20'42.57"	8°32'23.43"
PL01	41°21'41.56"	8°30'11.79"
AV12	41°20'46.91"	8°28'13.89"
VZ01	41°21'31.34"	8°25'45.54"
VZ06	41°22'18.11"	8°18'14.40"
SH07	41°27'58.60"	8°16'40.95"
AV27	41°29'1.59"	8°20'25.48"

The proximity to known sources of pollution, such as industrial facilities, was another critical factor. By strategically placing sampling stations near these potential polluters, the impact of their effluents on water bodies can be assessed. Moreover, the locations of the public water supply intakes were considered to ensure that any potential risks to drinking water sources could be accurately evaluated.

Accessibility is another critical factor. It is essential to select stations that are easily accessible for regular monitoring and sample collection. This facilitated the smooth operation of the monitoring program and ensured that data could be consistently gathered over time.

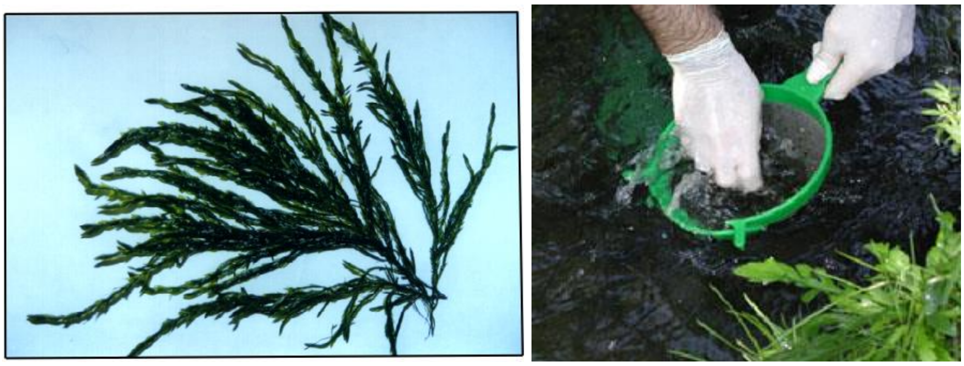
In terms of the chosen parameters, a comprehensive set of metallic ions, including Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Mercury (Hg), Nickel (Ni), Lead (Pb), and Zinc (Zn) were selected. These specific metals were deemed representative of the expected contamination levels, considering the types of industrial effluents typically discharged into the area.

3.3 ANALYTICAL METHODS

Moss samples (Fig. 2) were digested following the procedure described by Martins (2004) to analyse metals. The metal content was determined by atomic absorption spectroscopy (AAS). Cd, Cr, Cu, Fe, Ni, Pb, and Zn levels were determined using an air-acetylene flame on a GBC 902 spectrophotometer. Mercury was determined using a PYE UNICAM SP9 spectrophotometer with a UNICAM VP90 hydride generator.

Figure 2

Aquatic moss (Fontinalis antipyretica).



4 RESULTS AND DISCUSSIONS

Table 2 presents the metal amount per moss mass recorded at the 12 sampling stations in the Ave River basin for recent field surveys (campaigns II and III).

Based on the results, the highest metal accumulation in mosses was observed for Fe, with a concentration of 22424 µg g^{-1}. In contrast, the second sampling campaign found the lowest accumulation of Hg at a concentration of 1.6 µg g^{-1}. Analysing the most contaminated sites for each metal allowed the following accumulation order: Fe>Zn>Cu>Cr>Ni>Pb>Cd>Hg.

During Campaign II, relatively high levels of various metals were obtained at one or more stations in the Este River, except for Cr. Specifically, at station ES01, elevated concentrations of Cd, Cu, Ni, and Zn were recorded. At station ES04, elevated Cd, Cu, Ni, Pb, and Zn concentrations were observed. At station ES11, elevated Cu, Fe, and Hg concentrations were found. As for chromium, high values were registered at the Ave River (AV12), and iron occurred in the Pele and Pelhe Rivers (PH01 and PL01).

Table 2

Metal concentrations recorded at the sampling stations in the Ave River Basin.

Sampling Station	Concentration of metal in moss (□g g^{-1}), campaign II / III															
	Cd		Pb		Cu		Cr		Fe		Hg		Ni		Zn	
ES01	18	5	110	28	442	198	326	102	14176	6147	2.7	2.5	344	134	5932	1984
ES04	13	5	194	59	926	277	298	160	16608	7345	2.0	3.0	340	118	3373	907
ES11	8	5	125	<13	602	129	160	50	19956	7290	4.1	2.7	13	36	211	285
AV03	---	4	---	13	---	41	---	370	---	12455	---	---	---	42	---	586
AV04	9	4	104	<13	74	52	483	84	9359	5522	1.6	1.8	39	32	883	1102
AV12	9	5	58	<13	76	33	732	66	10180	3019	---	2.4	40	35	219	338
AV27	7	<4	63	<13	50	30	204	54	16962	7486	3.1	2.8	13	36	294	127
PH01	10	5	162	59	63	54	494	99	21689	13315	---	4.0	89	109	1169	876
PL01	8	4	68	<13	101	70	468	121	22424	12055	---	---	49	63	316	378
VZ01	9	5	104	13	112	72	334	82	8940	12981	---	---	26	62	963	278

VZ06	9	4	53	13	40	43	178	96	10075	10177	2.2	1.9	<13	63	143	316
SH07	7	5	62	13	38	42	330	31	10633	9455	1.7	2.1	<13	50	217	113

The substantial contamination observed in the Este River is most likely the result of untreated industrial effluent discharge, reflecting the metal coating facilities in Braga's urban area. The high chromium contamination recorded in Santo Tirso (AV12) is presumed to be a consequence of upstream discharges, specifically from tanneries and chromium plating industries along the Selho River.

During Campaign III, heavy rainfall occurred, leading to an increased river flow and reduced accumulation. Compared to the second campaign, the Este River continued to exhibit the highest levels of Cu, Ni, and Zn contamination. It is essential to highlight that stations ES01 and ES04 also exhibited Cr contamination. High contamination by Fe persisted at the mouths of the Pele and Pelhe rivers, and a new station, AV03, was added along the Ave River. AV03 exhibited the highest Cr concentration. In addition, there was an increase in Zn contamination in the Ave River at station AV04.

By comparing the results of campaigns II and III, it can be observed that there is an approximately linear relationship, with the values of the second campaign averaging 1.9 times those of the third campaign (Fig. 3).

As expected, contamination by heavy metals reflected the presence of metal coating, textile, and tanning industries in the area. When comparing the stations along the Este River, where the metal coating industry is highly prevalent, it is downstream from station ES11, where its effect is most prominent.

Water contamination at a specific location can be represented simply by a Contamination Factor,

$$F_c = \frac{CM}{CR} \qquad (1)$$

where:

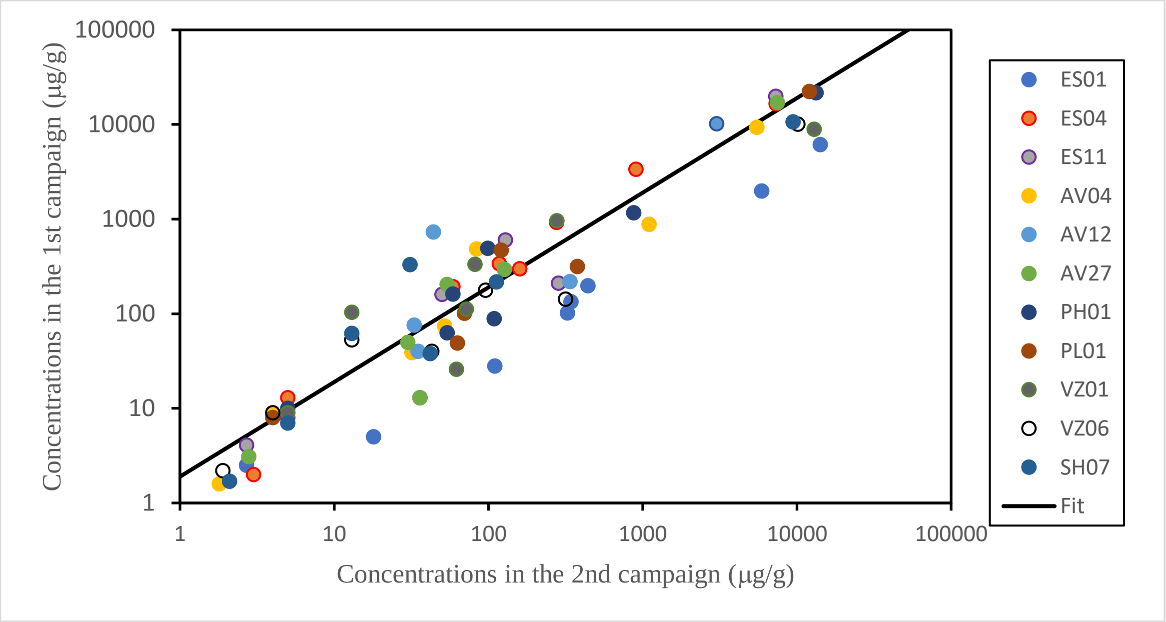
CM = concentration of metal in the moss collected at the location
CR = reference or natural concentration

Metal contamination exists at a given location for $F_c > 1$, whereas no contamination occurs when $F_c < 1$. Previous studies in the Ave Basin established reference concentrations for some metals (Gonçalves *et al.*, 1992; Branquinho *et al.*, 1994). The combination of these values with the minimum concentrations detected in this study resulted in the following reference concentrations (µg g^{-1}): Cd–4; Cr–30; Cu–30; Fe–3000; Hg–1.6; Ni–7; Pb–13; Zn–69.

Notably, all values (except for Cd, Cr, and Hg) were close to internationally adopted (Mouvet *et al.*, 1986).

Figure 3

Linear relation between the overall results obtained in the two campaigns.



A global qualitative classification can be conducted by assigning locations to Quality Classes using a new parameter called the Metal Pollution Index (MPI). This is calculated as the weighted average of various contamination factors for a specific location,

$$MPI = \sum(w_i F_{ci}) / \sum w_i \qquad (2)$$

where:

F_{ci} = contamination factor of metal i
w_i = weight of the ith metal

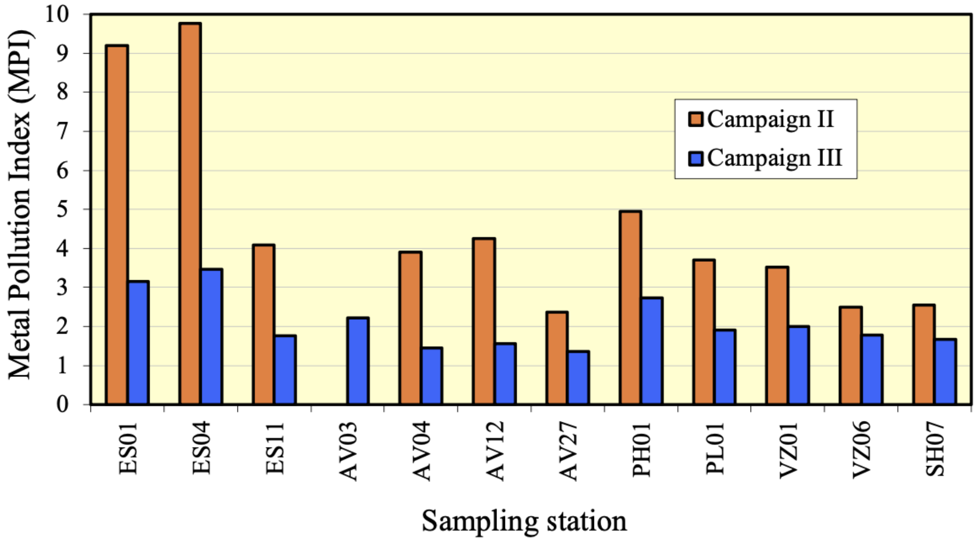
Under Portuguese and European Union legislation (Decree-Law No. 236/98), which considers the maximum permissible value (MPV) for each metal in surface waters intended for public water supply, considering their toxicity, weights were defined for the different metallic ions. It has been assumed that the toxicity of a particular metal varies inversely with its MPV. Therefore, the following weights were established:

$$w_{Cd} = 600;\ w_{Pb} = 60;\ w_{Cu} = 60;\ w_{Cr} = 60;\ w_{Ni} = 60;\ w_{Fe} = 10;\ w_{Zn} = 1 \tag{3}$$

Figure 4 shows campaigns II and III's Metal Pollution Index (MPI).

Figure 4

Metal Pollution Index for various sampling stations.



The sampling stations were classified into quality classes according to their Metal Pollution Index: Class A ($0 < MPI \leq 2$), Class B ($2 < MPI \leq 6$)), or Class C ($6 < MPI \leq 18$). At Class A sampling stations, contamination by the analysed heavy metals was either non-existent or insignificant. Class B represents moderate contamination, whereas Class C indicates significant contamination.

As previously mentioned, there was a significant difference in the contamination levels between the second and third campaigns. In Campaign II, only stations ES01 and ES04 showed a high level of pollution (Class C), whereas the remaining stations had moderate contamination (Class B).

Due to the impact of intense rainfall recorded in Campaign III, most stations decreased their classification. The two stations in the Este River moved down a level, now classified as

Class B, along with stations PH01 and AV03. The contamination detected at the remaining locations was deemed insignificant.

Figures 5 and 6 show the classification of the sampling stations according to the quality classes.

Figure 5

Distribution of sampling stations according to quality classes (campaign II).

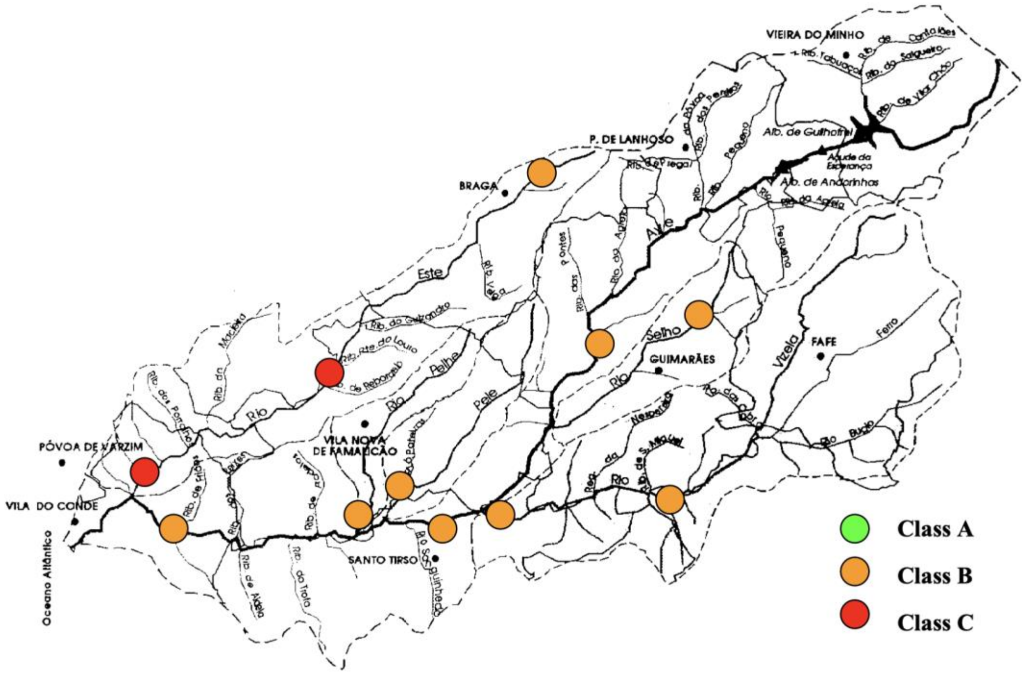


Table 3 compares the results obtained in recent campaigns (II and III) with those obtained for campaign I (Gonçalves *et al.*, 1992). Only the common metals and sampling stations between the two studies were analysed.

Based on the information provided, it can be concluded that chromium currently exhibits significantly higher values at all sampling stations compared to the first results. Additionally, considering only Campaign II, there was an aggravation regarding cadmium, lead, and zinc. However, based on the available data, no definitive conclusions can be drawn regarding copper.

Figure 6

Distribution of sampling stations according to quality classes (campaign II).

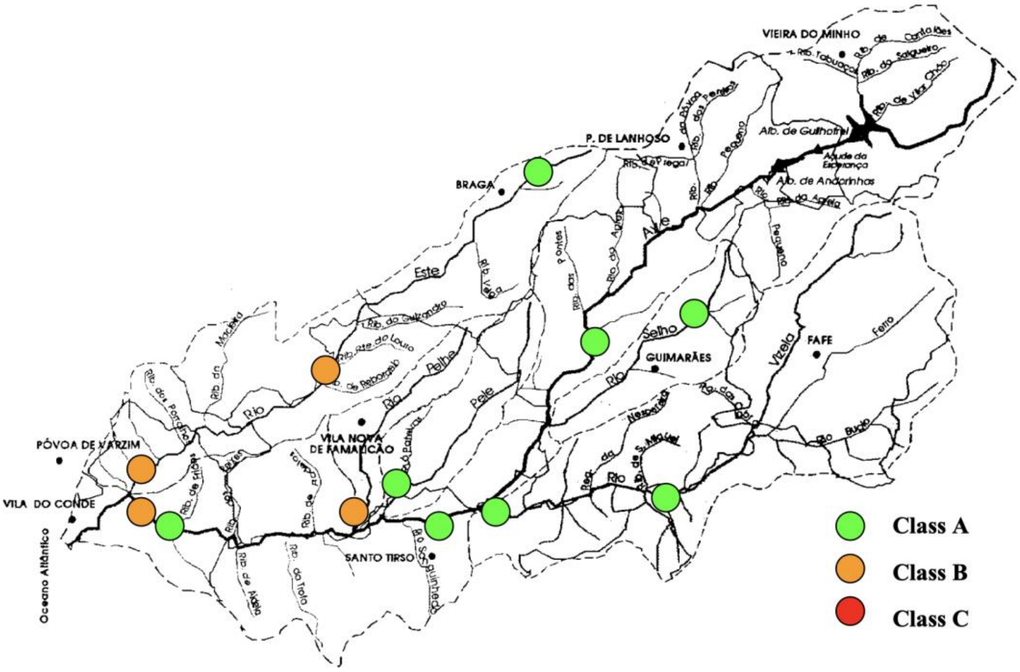


Table 3

Comparison between the current results and the results from Campaign I.

Sampling Station	Concentration of metal in moss (μg g^{-1}): campaigns I / II / III														
	Cd			Pb			Cu			Cr			Zn		
ES01	5.2	18	5	73	110	28	1496	442	198	38	326	102	4828	5932	1984
ES04	2.3	13	5	97	194	59	430	926	277	65	298	160	1427	3373	907
AV04	1.3	9	4	32	104	<13	34	74	52	23	483	84	86	883	1102
AV12	0.30	9	5	17	58	<13	49	76	33	30	732	66	112	219	338
PH01	0.64	10	5	38	162	59	77	63	54	7,5	494	99	585	1169	876
PL01	0.56	8	4	16	68	<13	167	101	70	14	468	121	154	316	378
VZ01	1.9	9	5	25	104	13	95	112	72	6,5	334	82	689	963	278
SH07	0.46	7	5	21	62	13	47	38	42	7.5	330	31	67	217	113

5 CONCLUSIONS

The analysis of metal concentrations in moss samples from the Ave River basin revealed several key findings. The highest metal accumulation in mosses was observed for iron (Fe), whereas the lowest accumulation was found for mercury (Hg) during Campaign II. The order of metal accumulation in the moss samples, from highest to lowest, was Fe > Zn > Cu > Cr > Ni > Pb > Cd > Hg. The contamination levels in the Este River suggest the influence of untreated industrial effluent discharge, mainly from metal coating facilities in Braga's urban area. High Cr contamination in Santo Tirso (AV12) is likely due to upstream discharges from tanneries and Cr plating industries along the Selho River. The third campaign, conducted during heavy rainfall, resulted in reduced metal accumulation and reclassification at several sampling stations. The Este River exhibited the highest Cu, Ni, and Zn contamination levels. Notably, stations ES01 and ES04 also exhibited Cr contamination. The Ave River (AV03) recorded the highest concentration of Cr, and there was an increase in Zn contamination at AV04. Metal Pollution Index (MPI): The classification of sampling stations into Quality Classes (A, B, or C) based on MPI revealed changes in contamination levels between the campaigns. Most stations experienced a decrease in classification due to increased water flow and reduced accumulation during Campaign III. A comparison of the results from the present study with those from campaign I (15 years earlier) revealed a significant increase in Cr levels. Additionally, aggravation of contamination was observed for Cd, Pb, and Zn. These findings highlight the ongoing presence of metal contamination in the Ave River Basin, with certain metals showing elevated levels and potential industrial sources affecting specific areas. Continuous monitoring and appropriate mitigation measures are essential to address and minimise the environmental impacts of heavy metal pollution in the region.

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