UNIVERZA NA PRIMORSKEM
FAKULTETA ZA MATEMATIKO, NARAVOSLOVJE IN INFORMACIJSKE TEHNOLOGIJE

MASTER THESIS
(MAGISTRSKO DELO)

PHYSICO-CHEMICAL AND ECOTOXICOLOGICAL EVALUATION OF MARINE SEDIMENTS QUALITY IN VICINITY OF COMMUNAL OUTLET CUVI: ROVINJ COASTAL AREA, NE ADRIATIC SEA, CROATIA

(FIZIKALNO-KEMIJSKA IN EKOTOKSIKOLOŠKA OCENA KAKVOSTI MORSKIH SEDIMENTOV V BLIŽINI KOMUNALNEGA IZTOKA CUVI: PRIOBALNO OBMOČJE ROVINJA, SV JADRANSKEGA MORJA, HRVAŠKA)

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Physico-Chemical and Ecotoxicological Evaluation of Marine Sediments Quality in vicinity of communal outlet Cuvi: Rovinj Coastal Area, NE Adriatic Sea, Croatia

(Fizikalno-kemijska in ekotoksikološka ocena kakvosti morskih sedimentov v bližini komunalnega iztoka Cuvi: priobalno območje Rovinja, SV Jadranskega morja, Hrvaška)

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Ključne besede: Morski sedimenti, Čistilna naprava, Kontaminacija, Smernice za kakovost sedimentov, Kemijske analize, Ekotoksikologija
Izvleček: Municipalne čistilne naprave za odpadne vode igrajo ključno vlogo pri ohranjanju javnega zdravja in zaščiti okolja z obdelavo in odstranjevanjem odpadnih voda in kanalizacije. Namen te naloge je bil ugotoviti potencialni vpliv municipalne obdelave odpadnih voda (UPOV Cuvi, Rovinj) na kakovost lokalnih morskih sedimentov (3 lokacije transek v bližini iztoka in kontrolna lokacija RV001) z fizikalno-kemijsko karakterizacijo (analiza velikosti delcev, vsebnosti vode in skupnega organskega ogljika, PCB-jev, 16 PAH-jev, težkih kovin) ter oceno potencialne toksičnosti (Pavg in Pmax po smernicah US EPA). Poleg tega so bili sedimenti predmet ekotoksikoloških analiz: Akutna toksičnost – Microtox test z *Vibrio fisheri*, kronična toksičnost – AlgalTox *Dunaliela salina* in fitotoksičnost - inhibicija kaljenja semen SG lax *Linum usitisimum*. Poleg tega, da bi ugotovili potencialni vpliv sedimentov na bioto in celotno oceno ekološkega tveganja, so bili upoštevani hrvaški (norveški) nacionalni pragovni vrednosti (Uradni list NN 28/2021), ERL (učinek nizkega razpona 10. percentil) - ERM (mediana učinka) vrednosti; vključno s ΣQN1 - kumulativnim tveganjem in QPECm – povprečnim tveganjem (francoske vrednosti N1 in N2) smernicami za kakovost sedimentov (SQGs). Rezultati kemijskih analiz preiskovanih morskih sedimentov, identificiranih kot osnovne vrednosti, so vse lokacije kategorizirali kot nepovzročena območja (I kategorija). Ocena nacionalnih in regionalnih SQGs je pokazala odsotnost potencialnih vplivov UPOV Cuvi na lokalno morsko okolje, kar dokazuje dober stanje okolja brez ekološkega tveganja za okolje in organizme na preiskovanih lokacijah. Z uporabo toksioloških testov (Microtox, AlgalTox in Fitotoksičnost) je bilo mogoče razlikovati sedimente kontrolne območja RV001 od sedimentov UPOV Cuvi (S7 – S9).

Key document information

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Keywords: Marine sediments, Wastewater treatment plant, Contamination, Sediment quality guidelines, Chemical analyses, Ecotoxicology
Abstract: Municipal wastewater treatment plants play a critical role in maintaining public health and protecting the environment by treating and disposing of wastewater and sewage. The aim of this thesis was to determine the potential impact of municipal wastewater treatment (UPOV Cuvi, Rovinj) on the quality of local marine sediments (3 sites-transect in vicinity of the outlet and control site RV001) by physico-chemical characterisation (analysis of grain size, water content and total organic carbon, PCBs, 16 PAHs, heavy metals), and potential toxicity assessment (Pavg and Pmax according US EPA). Further, sediments were subject of ecotoxicological analyses: Acute toxicity – Microtox test *Vibrio fisheri*, Chronic toxicity – AlgalTox *Dunaliela salina* and Phytotoxicity - Inhibition of seed germination SG lax *Linum usitisimum*. In addition, to determine the potential effect of sediments on biota and the overall ecological risk assessment, the Croatian (Norwegian) national threshold values (Official Gazette NN 28/2021), ERL (Effect of low range 10 percentile) - ERM (effect range median) values; including ΣQN1 - cumulative risk quotient and QPECm – average risk quotient (French N1 and N2 values) of the sediment quality guidelines (SQGs). Results of chemical analyses of investigated marine sediments identified as background values, categorized all sites as unpolluted areas (I category). National and regional SQGs evaluation indicated absence of potential UPOV Cuvi impacts to the local marine environment, proving a good state of the environment without ecological risk for the environment and organisms at the investigated locations. By applying toxicological test (Microtox, AlgalTox and Phytotoxicity) it was possible to differentiate sediments of the control area RV001 from sediments of UPOV Cuvi (S7 – S9) sites.

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LIST OF ABBREVIATIONS

AlgalTox - Algae chronic toxicity test
CECs - Contaminants of emerging concern
d.w. - Dry weight
DMSO - Dimethyl sulfoxide
EC50 - Effective concentration causing 50% inhibition
HCH - Hexachlorocyclohexane
Microtox - *In vitro* testing system which uses bioluminescent bacteria
PAHs - Polycyclic aromatic hydrocarbons
PCBs - Polychlorinated biphenyls
POPs - Persistent organic pollutants
SQGs - Sediment quality guidelines
TMs - Trace metals /Heavy metals
Tox - Toxicity
UPOV Cuvi - Wastewater treatment plant Cuvi
w.w. - Wet weight
w/v - Weight per volume

1. INTRODUCTION

1.1 IMPACT OF WASTEWATER TREATMENT PLANTS ON THE MARINE ENVIRONMENT

Municipal wastewater treatment plants (WWTP) play a critical role in maintaining public health and protecting the environment by treating and disposing of wastewater and sewage. However, these facilities also have the potential to impact the local marine environment if they are not operated properly. Municipal wastewater treatment plants can release various chemical contaminants into the marine environment. These contaminants may include pharmaceuticals, personal care products, industrial chemicals, heavy metals and microplastics. Although wastewater treatment plants are designed to remove many of these substances, not all are effectively removed, and traces can still enter the marine ecosystem (Freeman et al., 2020). The accumulation of these chemical contaminants in the aquatic environment can have harmful effects on marine organisms by disrupting their endocrine systems, interfering with reproduction, and even leading to bioaccumulation in the food chain. These contaminants can also end up in seafood consumed by humans, putting public health at risk (Silva, 2023).

One of the main impacts of municipal wastewater treatment plants on the local marine environment is nutrient enrichment. These facilities discharge treated wastewater into nearby water bodies, which often contain elevated levels of nutrients such as nitrogen and phosphorus. While these nutrients are essential for the growth of terrestrial plants, an excess of them in aquatic ecosystems can lead to harmful consequences such as algal blooms. Algal blooms reduce oxygen levels in the water, leading to hypoxia or "dead zones" where marine life struggles to survive. In addition, some algae species produce toxins that harm marine organisms and pose a risk to human health when seafood is contaminated.

The discharge of treated wastewater can release sediments into the marine environment. These sediments can smother sensitive habitats such as seagrass beds and reduce overall health and productivity. Sedimentation can also affect water clarity, which in turn affects the thriving of light-dependent organisms such as mussels, oysters, seagrasses and corals.

Wastewater treatment plants often discharge their effluents at different temperatures than the receiving waters, which can lead to thermal pollution. This can lead to temperature fluctuations that harm aquatic organisms, especially those that are sensitive to temperature fluctuations. Elevated water temperatures can also affect oxygen solubility in the water, which puts additional stress on marine life.

Microplastics, tiny plastic particles less than 5 mm in size, are a growing problem in the marine environment (Freeman et al., 2020). While municipal wastewater treatment plants focus primarily on the removal of larger wastes such as plastics and trash, microplastics can still pass through the treatment processes and enter the marine ecosystem. These microplastics can be ingested by marine organisms, which can lead to a range of ecological and organism vitality and health problems (Tamis et al., 2021).

1.1.1 Municipal wastewater treatment plant - UPOV Cuvi

The construction of Rovinj's sewerage network began in the 1980s, when the Cuvi wastewater treatment plant was built. The sewage network is regularly maintained and, if necessary, reconstructed and renovated by the local firm UPOV Cuvi - Odvodnja Rovinj-Rovigno d.o.o. The collector system with the Cuvi wastewater treatment plant with the underwater outlet into the sea covers the area of the town of Rovinj, the settlements of Rovinjsko Selo and Cocaletto, the southern tourist areas of Villas Rubin-Polari and Veštar and the northern tourist area of Monsena Valdaliso. To date, around 63 kilometres of sewage network have been built and the number of connections has risen to almost 5000. After pre-treatment at the UPOV Cuvi wastewater treatment plant, the partially treated wastewater was discharged through an 830-meter-long underwater outlet into the sea in the Cuvi area at a depth of 27 meters to the south. Due to the high population pressure – 12,000 people lived in the Rovinj catchment area in 2021, with a peak of 30000 in summer, and up to 700,000 tourists per year. From 2021 to 2023, UPOV Cuvi was rebuilt and upgraded with the third stage of wastewater treatment with a membrane bioreactor with a capacity of 63,000 population equivalents was built to achieve and maintain Good Environmental Status (GES). After the experimental work, beginning of the 2024 the underwater outlet is no longer in operation, and all the treated water are discharged to the mainland via canals further used to irrigate green areas of Rovinj city.

Municipal wastewater treatment plants are essential for the protection of human health and the environment. However, their operation can have unintended consequences for the local marine environment: chemical contamination, including nutrient enrichment, sedimentation, thermal pollution and microplastic pollution. To mitigate these impacts, it is crucial to implement advanced treatment technologies, improve monitoring and regulation, and promote public awareness and responsible consumption. By adopting sustainable practices and reducing the environmental footprint of wastewater treatment plants, we can minimize their negative impact on the marine ecosystem, ensuring a healthier future for marine life and our communities (Freeman et al., 2020).

1.1.2 Marine sediments

Marine sediments are an integral component of aquatic systems, linking multiple water uses, functions, and services. Contamination of sediments by chemicals is a worldwide problem, with many jurisdictions trying to prevent future pollution (prospective) and manage existing contamination (retrospective). Sediments are a suitable medium for studying pollution of the aquatic environment (Bihari et al., 2006; Pelikan et al., 2022). Actually, marine sediments represent a sink for a variety of contaminants over a period of time and can be used for marine quality-contamination assessment (Ruilian et al., 2008; Mamindy-Pajany et al., 2011). The sediments of the Mediterranean coasts e.g. harbours, marinas and industrial areas are particularly heavily contaminated with metals and organic compounds such as organotin compounds, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (Valković et al., 2007; Živković, 2010). Contaminated sediments can pose a serious threat to aquatic organisms and ecosystems, as they can release the contaminants back into the water column under certain conditions (Maher et al., 1999).

In the context of integrative environmental management, it is necessary to understand the natural and anthropogenic pressures on the marine ecosystem and the resulting environmental disturbances. The behaviour of contaminants in a marine ecosystem is the result of the interaction interplay of contaminants sources, physico-chemical properties of individual compounds, water and sediment movements, biotic and abiotic factors and specific conditions in marine areas, such as anoxia, eutrophication, freshwater springs and wastewater discharges. Determining the fate of contaminants in the marine environment is one of the most important steps in the risk assessment of marine habitats (Birch, 2018). After entering the marine environment, contaminants disperse in the water column and accumulate in sediments and biota. The assessment of the environmental impact of contaminants is based on the determination of the concentration of contaminants in the relevant matrix and the impact of the contaminants on species and habitats (MSFD, 2008).

Chemical analyses of specific contaminants provide information about their input and possibly their source. However, the relationship between the presence of certain contaminants, their distribution in different matrices and their ability to affect marine organisms is very complex. Contaminants in the marine environment include a range of compounds and form complex mixtures that may or may not be toxic. The toxicity of samples from polluted environments can be caused by hundreds of different compounds, and often there is no correlation between a particular contaminant load and toxicity (Bihari et al. 2006; 2007; Linšak et al., 2012; Glad et al., 2017).

1.2 MAIN TYPES OF CONTAMINANTS

1.2.1 Organic compounds

1.2.1.1 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous widespread contaminants arising mainly from incomplete combustion of fossil fuels, organic materials, wood and petroleum. PAHs enter marine coastal areas through spillages, industrial discharges, atmospheric fallout and urban runoff (Neff, 1979). Due to their low solubility in water and their hydrophobicity PAHs rapidly become associated with organic and inorganic suspended particles (Chiou et al., 1998) in the marine environment and subsequently deposited in sediments. These sedimentary PAHs have a tendency to accumulate to high concentrations, however, levels may vary depending on site characteristics, biodegradation and proximity to sites of human activity. Some PAHs are known to be toxic and increase the toxic potency of sediments (Shor et al., 2004) and some certain PAHs and their metabolites affect DNA (Singh et al., 1998) and have carcinogenic properties (IARC, 2015). They are also recognised as progenotoxic contaminants.

1.2.1.2 Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are a group of persistent organic pollutants (POPs), the term referring to a class of 209 chlorinated biphenyl isomers that were widely used in numerous industrial applications from 1929 to the late 1970s. PCBs can be unintentionally produced during combustion, chlorination of water, and different industrial production processes. PCBs are capable of exhibiting ecotoxicity effects far away from their point of emission due to their ability to undergo long distance migration, their environmental stability, lipophilicity and capacity to bioaccumulate and biomagnify. PCBs are hydrophobic with low solubility in water, but high solubility in organic solvents, oils and fats (UNEP Chemicals, 1999). Despite being hydrophobic they accumulate mostly in the hydrosphere inside the organic fraction of soil and in organisms. The main contributor of PCBs in organisms are microplastics (Ma et al., 2019). In the aquatic setting, PCBs are adsorbed onto particulate matter, and undergo sedimentation processes and, consequently, build up in sediments. Sediment has a high retention capacity for PCBs, owing to its surface area, and can be a secondary source of PCBs to the overlying water body through mechanical or physical turbation or changes in the geochemical properties of the sediments (Sakan et al., 2017). Therefore, sediment plays a significant role in determining the fate and global cycling of PCBs.

1.2.2 Trace metals

Trace (heavy) metals (TMs) are natural constituents of the marine environment and are normally found in very low concentrations. Human activity has increased natural levels of metals through various activities: mine drainage, oil and gas exploration, industry (paints, pesticides, pharmaceuticals etc.), agricultural runoff and domestic effluents.

Heavy metals are considered a major anthropogenic contaminant in coastal and marine environments worldwide (Ruilian et al., 2008). They pose a serious threat to human health, living organisms and natural ecosystems because of their toxicity, persistence and bioaccumulation characteristics (DeForest et al., 2007). Anthropogenically, heavy metals can be introduced to coastal and marine environments through a variety of sources, including industries, wastewaters and domestic effluents (Fu and Wang, 2011). Metals released to marine environments rapidly bind to particulates and sink to the sea bottom (Hedge et al., 2009). Marine sediments, consequently, act as an ultimate sink for heavy metals input into the aquatic environments (Ruilian et al., 2008).

1.2.3 Emerging CECs - New compounds

Contaminants of emerging concern (CECs) are pollutants that have been detected in environmental monitoring samples, that may cause ecological or human health impacts, and typically are not regulated under current environmental laws. Sources of these pollutants can include agriculture, urban runoff and ordinary household products, and pharmaceuticals that are disposed in sewage treatment plants and subsequently discharged to surface waters. Examples of emerging contaminants are 1,4-Dioxane, food additives, pharmaceuticals, and natural & synthetic hormones (Feng et al, 2023). A newly emerging issue can be considered the possibility of antibiotic resistant bacteria and other microorganisms that could potentially cause harm to humans after adapting to antibiotics due to exposure to low concentrations present in sewage and drainage (Vassallo et al., 2021).

1.2.4 Microplastics

Microplastics are small particles and fibres of plastic. There is no recognised standard for the maximum particle size but they are generally considered to be particles measuring less than 5 millimetres in diameter, a classification that includes nano-size plastics which are fragments measuring less than 100 nanometres. They are largely classified by their morphological characteristics: size, shape and colour. Size is in particular an important factor when studying microplastics as it dictates the range of organisms it may affect (Bošković et al., 2021). Microplastics are introduced into the marine environment in various ways: shards form vehicles and clothing, industrial and household runoff, from the atmosphere via land-originating wind-carried particles, and the degradation of larger plastic debree. These particles can cause significant issues due to their capability of bioaccumulation and biomagnification, causing a wide range of symptoms in marine organisms depending on their size. The smallest can affect metabolisms while the largest can cause mechanical issues, like inhibiting muscle motion due to their sheer size or clogging the stomachs of individuals. Microplastics are also significant in that they are a suitable substrate (adhesion) for other possibly harmful substances persisting in the marine environment, as well as a medium for transporting substances further from their original source and spreading, introducing substances into other organisms (Freeman et al., 2020).

1.3 ACCUMULATION OF CONTAMINANTS IN MARINE SEDIMENTS

Sediments are an integral component of aquatic systems, linking multiple water uses, functions, and services. Contamination of sediments by chemicals is a worldwide problem, with many jurisdictions trying to prevent future pollution (prospective) and manage existing contamination (retrospective) (Ridgway and Shimmield, 2002). Sediments are considered a suitable medium for studying pollution of aquatic environments because they represent a sink for a variety of pollutants over a period of time (**Figure 1**).

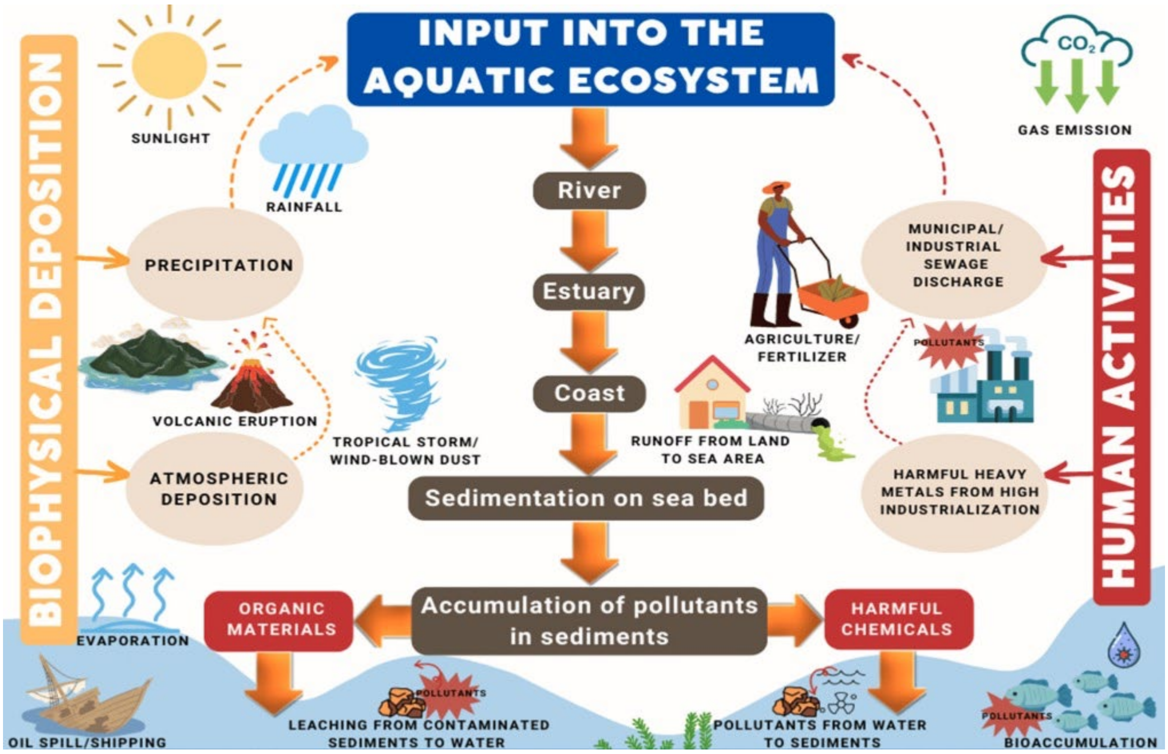

Figure 1: Marine sediments as suitable medium for studying pollution of the aquatic environment.

Contamination of sediment presents a significant risk to the health of benthic organisms, that is, reduction in survival, growth (Baker and Kravitz, 1992; Krahn and Stein, 1998), and reproduction (MacDonald et al., 2003). Contaminated sediment impairs the development of vegetation and bacteria and, because these organisms are the foundation of the food chain, these chemicals affect other forms of aquatic life (MacDonald et al., 2003). Sediment is necessary for protection and shelter of juveniles and is vital to many organisms for spawning during breeding (MacDonald et al., 2003). Adverse effects are observed in higher trophic organisms through direct contact with contaminated sediment and through consumption of contaminated organisms.

1.4 SEDIMENT QUALITY GUIDELINES

Sediment quality guidelines (SQGs) are an important tool for assessing pollution of marine and estuarine sediments (Kwok et al., 2014). Although such guidelines are not definitive indicators of toxicity, they can be highly predictive and useful for identifying areas of potential adverse biological impact. Despite the fact that the weight-of-evidence (WOE) approach was developed in combination with multiple lines of evidence (LOEs) for sediment characterization, some regulatory frameworks still rely on chemical characterization relative to SQGs as stand-alone decision criteria (Chapman and Hollert, 2006; Dagnino et al., 2008; Benedetti et al., 2012).

1.4.1 International

Different approaches for deriving sediment quality criteria are widely used in Europe, the USA, Canada and Australia (Carere et al., 2008). For example, the sediment quality guidelines proposed by Long (2006) and the US EPA (2005) based on biological toxicity tests of the benthic environment (Liao, 2021) were developed as a composite indicator divided into three levels with effect range low (ERL) and effect range median (ERM) values (**Table 1**).

In environmental toxicology, ERL and ERM are measures of toxicity in marine sediments. They are used by public agencies in the United States in formulating guidelines for assessing toxicity hazards, particularly from trace metals or organic contaminants (US EPA, 2005). The ERL and ERM measures are expressed as specific chemical concentrations of a toxic substance in sediment. The ERL indicates the concentration below which toxic effects are rarely observed or predicted; the ERM indicates the concentration above which effects are generally or always observed (Long et al., 1995). They are not regulatory criteria and should not be used as such. The US EPA uses ERL and ERM values as a type of "benchmark" for sediments. They define a benchmark as a concentration that, if exceeded, has the potential to cause harm or significant risk to humans or animals in the environment.

Table 1: Sediment Quality Guidelines threshold ERL - ERM (NOAA) values, and TEL - PEL (FDEP) values for Metals (mg/kg d.w.), Metalloids (mg/kg d.w.) and Organics (µg/kg d.w.) used for sediment categorisation.

Parameters	ERL – Effect of low range (10 percentile)	ERM – Effect of median range (50 percentile)	TEL – Threshold effect level	PEL – Probable effect level
As	8.20	70.00	7.24	41.60
Cd	1.20	9.60	0.68	4.21
Cr	81.00	370.00	52.30	160.00
Cu	34.00	270.00	18.70	108.00
Hg	0.15	0.71	0.13	0.70
Ni	20.90	51.60	15.90	42.80
Pb	46.70	218.00	30.20	112.00
Zn	150.00	410.00	124.00	271.00
Total PAHs	4,022.00	44,792.00	1,684.00	167,770.00
Total PCBs	22.70	180,00	21.60	189,00

Another effects-range approach developed by MacDonald et al. (1996; 2000) uses a greatly expanded biological effects database for sediments normalized to 1% organic carbon. Biological effects data were again sorted for a particular contaminant into two separate sets, that is, concentrations that produced a biological effect and those that had no adverse effect. From the no-effects data, the 50th percentile (No Effect Range Median, NER-M) and the 85th percentile (No Effect Range High, NER-H) were calculated. The effects data were also sorted and the lower 15th percentile (ERL) and median or 50th percentile (ERM) determined. The threshold effects level (TEL) defined the upper limit of sediment contaminant concentrations of data with no effects, that is, >75% no-effects data, which was calculated as the geometric mean of the ER-L and NER-M.

$$TEL=(ERL\times NER\text{-}M)1/2$$

The no-observed-effects level (NOEL) was defined as the TEL multiplied by a safety factor of 2.

The probable effects concentrations (PEL) defined the lower limit of the range of contaminant concentrations that were normally associated with adverse biological effects, that is, > 75% effects data, which was defined as the geometric mean of the ER-M and NER-H values:

$$PEL=(ERM\times NER\text{-}H)1/2$$

Assessments of these approaches showed that ERMs and ERLs were generally as reliable as PELs and TELs in classifying samples as toxic or nontoxic (Long and MacDonald, 1998). Both guidelines provide two values for each chemical which identifies sediment which is rarely (<ERL, or <TEL), occasionally (>ERL/TEL<ERM/PEL), or frequently (>ERM, or PEL) associated with adverse biological effects.

1.4.2 Regional

Even at the EU level, regulations and types of sediment analysis vary from country to country, and the European Commission does not require Member States to adopt a particular approach (Röper and Netzband, 2011). In general, the chemical criteria with national threshold values approach should be supported by bioassays, bioaccumulation tests and ecological analyses to define an integrated assessment of contaminated sediments and better evaluate toxic effects on benthic organisms and impacts on aquatic ecosystems. Such assessment frameworks should be based on broad consensus and be flexible and adaptable as new data become available.

1.4.2.1 Italy

The neighbouring country of Croatia, Italy, uses integrated WOE criteria (Italian Ministry of Environemntal Decree, 2016). Such multidisciplinary WOE approaches include integration of different lines of evidence (LOEs): 1. chemical characterization of sediments; 2. bioavailability of chemicals; 3. sublethal effects—biomarkers; 4. ecotoxicological bioassays; 5. analysis of benthic communities after classification into WOE risk classes (absent, slight, moderate, major, and severe) (Pane et al., 2008; Benedetti et al., 2012; Piva et al., 2011; Onorati et al., 2017; Avio et al., 2017). Considering the triad of sediment quality, a consensus on full chemical and ecotoxicological characterization and WOE risk assessment of (contaminated) sediments should balance availability, financial costs, scientific benefits and cost advantages (Chapman, 1990; Hamer et al., 2019).

1.4.2.2 France

The French sediment quality guidelines (SQGs) are using two guide levels (N1 and N2) for the concentrations of selected metals (As, Cd, Cu, Ni, Pb, Zn, Hg and Cr), ΣPAHs, and ΣPCBs in the total sediment (JORF No. 184, 10-08-2000). According to this decree the sediment is considered uncontaminated if the pollutant concentration is below the threshold N1. If the pollutant concentrations are between the N1 and N2 thresholds, the sediment is classified as contaminated, and the associated ecological impacts must be evaluated. If at least one contaminant is above the N2 threshold, the sediment is considered highly contaminated with potential ecological impact on the aquatic environment. The N1 and N2 thresholds can be derived mainly from statistical processing of physical and chemical data.

Further the calculated ∑QN1 and QPECm risk quotients are used as appropriate indicators of potential ecological effects on the aquatic environment. ∑QN1 represents the sum of all individual ratios between the contaminant concentration and the French N1 legal level (PEC value). The general sediment risk quotient QPECm represents the averaged ∑QN1 value for a given sediment divided by the number of all pollutants studied, taken from the French guidelines for the management of marine dredged sediment (Alzieu and Quiniou, 2001; Perrodin et al., 2006; Mamindy-Pajany et al. 2011; Pelikan et all. 2022). In addition, for assessing the potential ecological impact of contaminated sediment classes II and III, the decree proposes a simple determination of acute/chronic toxicity using standard tests (microtox, algaltox and phytotox), loosely linked to EU risk assessment principles.

1.4.3 National – EU MFSD

The Marine Strategy Framework Directive - MSFD (European Commission, 2008b) is an equivalent directive to the WFD for marine waters, aiming to achieve and maintain a good environmental status (GES) in the marine environment. The qualitative descriptors for determining GES include contaminant concentrations in fish and other seafood at levels safe for human consumption. Member states should assess compliance with the EQSs set for the coastal waters (marine) in the WFD and may set threshold values (national EQSs) for other substances in a specific matrix, like sediment. There are no mandatory directive-level guides or requirements for using sediment bioassays while assessing environmental status, but biological approaches are included as supplementary criteria to assess GES. Some member states evaluate compliance with the MSFD using several biomarkers in fish, mussels, birds, and benthic biota. When implementing the MSFD and determining GES of marine waters, member states are recommended to collaborate within each marine region or subregion, for example, by using Regional Sea Conventions. The Helsinki Commission (Baltic Marine Environment Protection Commission) is an example of a region-specific action. For its GES assessment, the Baltic Sea Action Plan (HELCOM, 2021) uses concentrations of several core indicator contaminants measured in biota or sediment. Countries around the Baltic Sea have agreed with HELCOM on threshold values representing a GES (e.g., for copper and tributyltin in sediments). Some biological endpoints are also used as biomarkers (e.g., imposex, malformed amphipod embryos); but these are not formerly assessed (HELCOM, 2023), and specific whole- sediment bioassays are not applied. On another multinational level, the Oslo–Paris (OSPAR) Convention (1992) promotes ecosystem approaches through the concept of ecological quality objectives (i.e., a large set of operational objectives and indicators to assess health of marine ecosystems), providing the impact assessment of found pressures. A two-stage process is used that compares bioassay results to environmental assessment criteria and then against background (assessment) concentrations.

Croatian legislation relating to marine sediments is covered in particular by the Marine Strategy Framework Directive, the Water Framework Directive, the Urban Waste Water Treatment Directive and national Sediment Quality Guidelines (Action Programme for the Management of the Marine Environment and Coastal Area Strategy - Monitoring and Observation System for the Permanent Assessment of the State of the Adriatic Sea 2021 – 2026: Indicator D8 – Contaminants; D8.C1 Concentrations in sediments, Metals, PAHs, PCBs; D8.C2 sediment impact on biota, *Mytilus galloprovincialis* and *Vibrio fischeri* (**Table 2**).

Table 2: Croatian criteria for sediment quality and contamination assessment (MFSD Official Gazette 28/2021) actually use Norwegian criteria (Bakke et al., 2010).

Sediment Category Pollution	**I (Background)**	**II (Good)**	**III Moderate**	**IV (Bad)**	**V (Very bad)**
(mg/kg d.w.)	**Background levels**	**No toxic effects**	**Toxic effects following chronic exposure**	**Toxic effects following short term exposure**	**Severe acute toxic effects**
As	<20	20-52	52-76	76-580	>580
Cd	<0.25	0.25-2.6	2.6-15	15-140	>140
Cr	<70	70-560	560-5,900	5,900-59,000	>59,000
Cu	<35	35-51	51-55	55-220	>220
Hg	<0.15	0.15-0.63	0.63-0.86	0.86-2	>1.6
Ni	<30	30-46	46-120	120-840	>840
Pb	<30	30-83	83-100	100-720	>720
Zn	150	150-360	360-590	590-4,500	>4,500
Total PAHs	<0.30	0.30-2.00	2.00-6.00	6.00-20.00	>20.00
Total PCBs	<0.005	0.005-0.017	0.017-0.190	0.190 -1.900	>1.900

1.5 ECOTOXICOLOGY

European regulations in which sediment toxicity testing is conditionally required as part of substance safety assessments or for registration purposes include the European Regulation (EC) 1907/2006 (European Commission, 2006) concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and 1107/2009, which lays down procedures for authorization of plant protection products. The REACH regulation requires sediment toxicity information for substances produced or imported in quantities ≥1,000 t/y based on substance properties (Leppanen et al., 2024).

Chemical data on concentrations and mixtures of contaminants alone do not provide an effective basis for determining the potential adverse effects on living resources. Information on persistence, toxicity, and bioaccumulation is required to establish the biological significance of sediment-bound contaminants. These tests are expensive and require a high level of expertise; hence, SQGs are commonly used to make an initial assessment of sediment toxicity in the absence of direct biological effects data (Batley, and Simpson, 2013). The chemical status assessment of the aquatic environment in the European Union is mainly based on concentrations of pollutants, and biological approaches are only voluntary. The European Commission has published guidance to enhance harmonization among member states in chemical monitoring of sediment and biota (European Commission, 2000). This guidance document focusses on chemical monitoring but also briefly introduces complementary methods, stating “bioassays, biomarkers and other ecotoxicological tests are useful tools for the evaluation of the real state of sediment in which both known and unknown contaminants are present at concentrations sufficient to cause toxicity to the test organisms." A triad approach (i.e., chemical, bioassays, ecology), TIE, and effect-directed analysis (i.e., the identification of toxic, causative organic contaminants by a combi- nation of sample fractionation, bioassays, and chemical analyses) are mentioned as useful approaches; but specific methods are not described. In addition, international treaties like HELCOM, OSPAR, and the Barcelona Convention locally regulate and guide dredging and disposal. Nationally, many member states predominantly use contaminant threshold values for assessing the risks and relocation solutions for dredged material (Heise et al., 2020). The use of bioassays appears to be very limited in Europe, but some guidelines and practices are in use (e.g., Italy, France, Germany) or have been part of regulations in the past.

2. AIM AND HYPOTHESES

2.1 AIM

The aim of the thesis was to determine the potential impact of municipal wastewater treatment plant (UPOV Cuvi, Rovinj) on the quality of local marine sediments (3 sites-transect in vicinity of the outlet and control site RV001) by physico-chemical characterisation (analysis of grain size, water content and total organic carbon, PCBs, 16 PAHs, heavy metals), and potential toxicity assessment (Pavg and Pmax according US EPA). Further, sediments were subject of ecotoxicological analyses: Acute toxicity – Microtox test *Vibrio fisheri*, Chronic toxicity – AlgalTox *Dunaliela salina* and Phytotoxicity - Inhibition of seed germination SG lax *Linum usitisimum*. In addition, to determine the potential effect of sediments on biota and the overall ecological risk assessment, the Croatian (Norwegian) national threshold values (Official Gazette 28/2021), ERL (Effect of low range 10 percentile) - ERM (effect range median) values (Macdonald et al, 2000); including ΣQN1 - cumulative risk quotient and QPECm – average risk quotient (French N1 and N2 values) of the sediment quality guidelines (SQGs) were used.

2.2 HYPOTHESES

1. The input of the UPOV Cuvi municipal wastewater treatment plant has an impact on the chemical contamination of sediments at the investigated sites and at the local marine environment.
2. The concentrations of possible chemical contaminants (PAHs, PCBS, TMs) increase along the studied transects (50, 200, 1000 m) in front of the UPOV Cuvi outlet.
3. The contaminants concentrations are positively corelated with the results of the toxicity assays, which all reflect the contaminant load of the selected sites.
4. The analyses of chemical contaminants in the sediments evaluated according national and regional sediment quality guidelines, are allowing the sites to be ranked and classified, according environmental status and ecological risk for local organisms.
5. The integration of sediment eluates/extracts toxicity test results with the analyses of chemical contaminants in the sediments will further dis/approve the impact of the UPOV Cuvi outlet discharge on the local marine environment.
6. The potential toxicity of sediments depends on the sediment type.

3. MATERIALS AND METHODS

3.1 INVESTIGATED AREA

Four sampling sites were investigated in the coastal waters in eastern Adriatic Sea near the city of Rovinj City with 12,000 inhabitants (**Figure 2**). The sampling sites differ according possible anthropogenic impacts. Sampling sites are selected according to the Study of environmental impact (SUO) UPOV Cuvi (Elaborat zaštite okoliša, 2015) and previous contamination results (Ušić and Travizi 2011; Paliaga, 2015; Paliaga et al., 2017), taking into account the history of contaminations and environmental activities in the area. The UPOV Cuvi underwater outlet is 830 m from the coast and is located at a depth of 27 m to the south. The seabed near the Cuvi outlet was characterized as fine-grained sediment, i.e. sandy silt. Marine currents run in a north-westerly direction, while in the bottom layer the northern component of the current predominates with weak intensity.

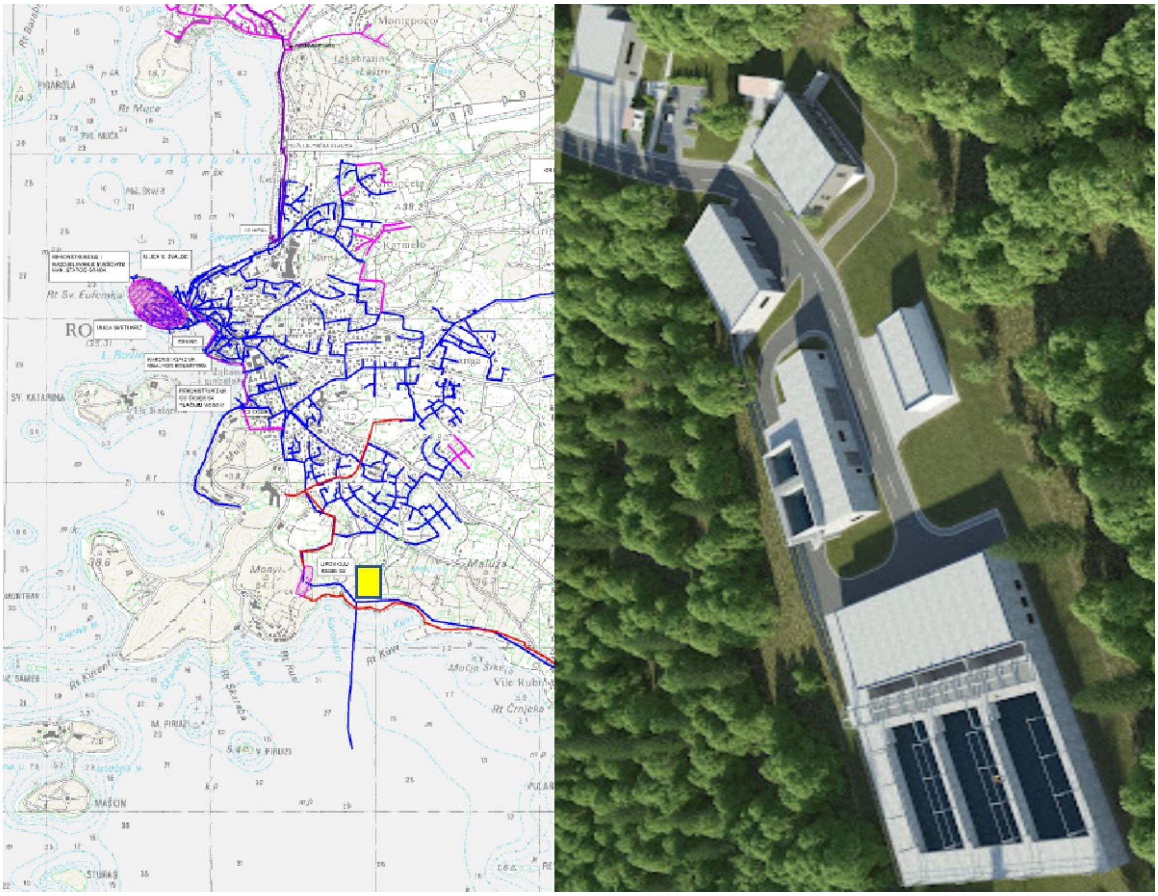

Figure 2: Wastewater treatment plant UPOV Cuvi with collection network scheme of Rovinj coastal area.

3.2 MATERIALS

3.2.1 Chemicals

Cyclohexane (Merck, Darmstadt, Germany); Silica gel (Kemika, Zagreb, Croatia); Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Darmstadt, Germany); PAHs internal standards (Supelco, St. Louis, USA).

3.3 METHODS

3.3.1 Sediment sampling

The upper layer of surface sediments (< 5 cm) was collected in May 2022 using a Van Veen grab (0.25 m^2 mouth area) from a boat at the control/reference site (RV001) 1 NM from the town of Rovinj (GPS 45°04′.808 N; 013°36′.541 E; depth 25 m) and at sites 50 m (45°03′.488 N; 013°38′.763 E; depth 24 m), 200 m (45°03′.312 N; 013°38′.770 E; depth 27 m) and 1000 m (45°02′.954 N; 013°38′.822 E; depth 29 m) from the UPOV Cuvi outlet (**Figure 3**).

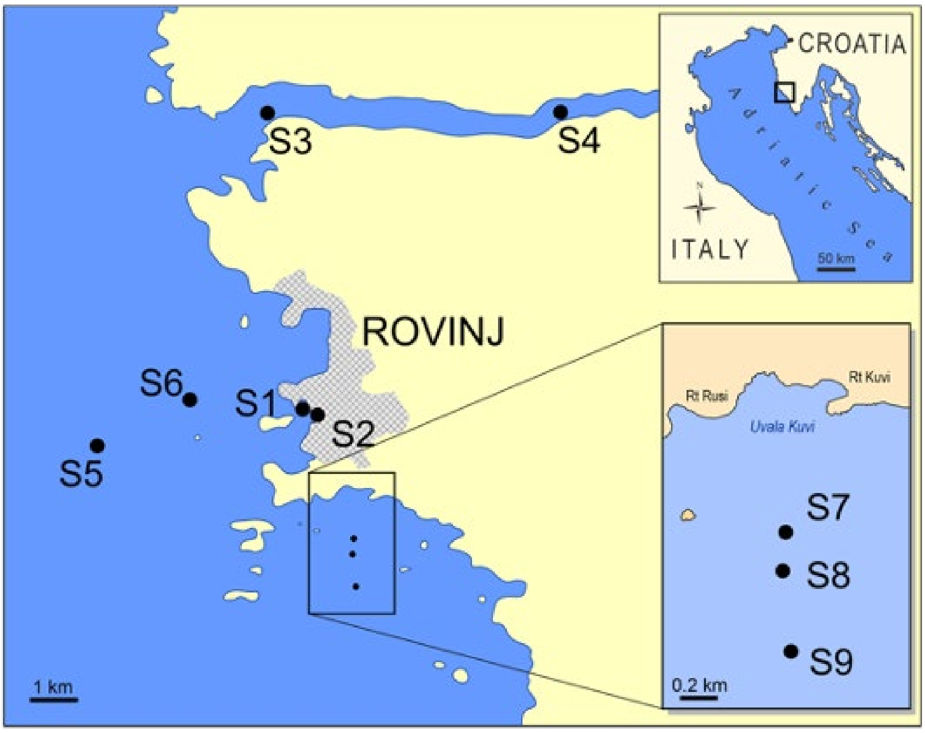

Figure 3: Map of investigated area with sampling sites locations in vicinity of UPOV Cuvi: S7 (50 m), S8 (200 m), S9 (1000 m) and S6 reference site (RV001).

3.3.2 Physico-chemical characterisation

3.3.2.1 Grain size analysis

The top 5 cm of undisturbed sediment from each sediment sample was carefully subsampled through the top window of the grab. Prior to further subsampling, the collected 5 cm surface sediment was carefully homogenized with a firm plastic spoon. The homogenized sediment samples were stored at +4°C immediately after sampling and then stored at −80°C in the laboratory until analysis. Prior to grain size analysis, sediment samples were defrosted and weighed. Their dry weight was calculated as the weight loss after drying at 70°C for 24 h. Wet sieving method with Retsch sieves was used to separate coarse-grained (>0.063 mm) and fine-grained fractions (<0.063 mm). The coarse-grained fractions were dried and further dry-sieved using a set of 7 sieves with the following mesh sizes: 4 mm, 2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.125 mm and 0.063 mm. The mud fraction was not further separated into silt and clay. The sediment retained on each sieve was dried and weighed, while the weight of the fine-grained (mud) fraction was calculated using the initial dry weight. The GRADISTAT statistical package (Blott and Pye, 2001) was used for the basic granulometric description: sediments were classified according to the Folk scheme (Folk et al., 1954; Buchanan & Kain, 1971).

3.3.2.2 Water content

The water content of the sediments was determined by sediment heating (50 g) at 105°C for 24 hours and weighting dry weight in Incubator (ST-05 Instrumentaria; Tvornica Medicinskih Instrumenata aparata i šprica, Zagreb, Croatia).

3.3.2.3 Total organic carbon content

The presence of total organic material (TOC) was estimated by weight loss after heating of sediments (10 g) at 450°C for 5 hours in Combustion device (INKO 1935, Zagreb, Croatia).

3.3.3 Polycyclic aromatic hydrocarbons

The Polycyclic aromatic hydrocarbons (PAHs) were analysed and quantified by the Teaching Institute of Public Health of Primorsko-goranska County, Rijeka, as previously described (Pelikan et al., 2022). The previously dried sediment (10 g) was extracted with 100 mL of spectrograde cyclohexane (Merck, Darmstadt, Germany) in an ultrasonic bath for 45 minutes. After filtration, the extracts were evaporated to dryness using a rotary evaporator. The residue was dissolved in 1 mL cyclohexane and the PAH fraction was separated on a column with 1 g silica gel (Kemika, Zagreb, Croatia) and some Al_2O_3 on top. The column was eluted with 25 mL of cyclohexane and the eluate evaporated almost to dryness on a rotary evaporator. The remaining PAH fraction will be dissolved in 1 mL of distilled methanol and analysed by HPLC (Linšak et al., 2012).

Sixteen PAHs; Naphtalene (1.00 ng/kg detection limit), Acenaphthylene (1.00 ng/kg detection limit), Acenaphthene (1.00 ng/kg detection limit), Fluorene (1.00 ng/kg detection limit), Phenanthrene (1.00 ng/kg), Anthracene (1.00 ng/ kg), Fluoranthene (2.00 ng/kg), Pyrene (1.00 ng/kg), Chrysen (1.00 ng/kg), Benzo(a)anthracene (1.00 ng/kg), Benzo(b)fluoranthene (1.00 ng/kg), Benzo(k)fluoranthene (1.00 ng/kg), Benzo(a)pyrene (1.00 ng/kg), Dibenzo(a,h)anthracene (1.00 ng/kg), Bcnzo(g,h,i)perylene (1.00 ng/kg) and lndeno(l,2,3-c,d)pyrene (1.00 ng/kg), were identified and quantified using Supelco internal standards using an HPLC module system HP 1050 (Hewlett Packard, Palo Alto, CA, USA). This system consisted of a quaternary pump with a rheodine valve injector (HP 7125) and a 20 nL sampling loop with a variable wavelength UV detector and integrator (HP 3396) as previously described (Linšak et al., 2012). PAHs were separated on a reversed phase cartridge column HP Li Chrospher 100 RP-18 (51 m, 25 x 4 mm).

3.3.4 Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) were analysed and quantified as Aroclor 1260 by the Teaching Institute of Public Health of Primorsko-goranska County, Rijeka, as previously described (Pelikan et al., 2022). Approximately 20 g of wet sediment was homogenised with anhydrous $NaSO_4$ and extracted with n-hexane according to Linšak et al (2012). The extracts were analysed with GC/MS–QP2010 (Shimadzu Corp., Kyoto, Japan) equipped with an AOC-5000 autoinjector (CTC Analytics AG, Twingen, Switzerland).

3.3.5 Heavy metals

Heavy metals (As, Cd, Cu, Ni, Pb, Zn, Hg and Cr), called trace metals (TMs) also, were analysed and quantified by the Teaching Institute of Public Health of Primorsko-goranska County, Rijeka, as previously described (Linšak et al., 2012). Sediment samples were dried overnight in an oven at 105°C. The samples (1g, < 2 mm grain size fraction) were heated with HNO_3 in a microwave oven (PerkinElmer/Anton Paar Multiwave 3000). The metal content was determined using a Perkin Elmer (Waltham, MA, USA) 4110 ZL (Pb, Hg and Cd) and a Perkin Elmer Analyser 200 (Cu and Zn). Pb and Cd were determined using the graphite furnace atomic absorption spectroscopy (GFAAS) method, while Hg was determined using the flow injection mercury system (FIMS) method. Cu, Zn and the other metals were determined by direct flame atomization.

3.3.6 Acute toxicity on bacteria *Vibrio fisheri* – Microtox®

The sediments (50 g wet weight) were extracted with dichloromethane–methanol (2:1) as previously described (Schiewe et al., 1985). After washing several times to remove the methanol, the dichloromethane extracts were evaporated to dryness and the extracts were dissolved in 100 µl dimethyl sulfoxide (DMSO) solvent for wide range of non-polar to polar compounds. Extraction blanks were prepared using the same procedure, but without sediment. The sediment extracts were stored at 4ºC prior to the toxicity tests.

The toxicity of the organic extracts from sediment were measured using the Microtox® bioassay according to Bihari et al. (2007). The Microtox bioassay measures the decrease in bacterial luminescence (*Aliivibrio fisheri*) after exposure to a series of dilutions of the samples according to the BioFix® Lumi procedure prescribed by the manufacturer Macherey-Nagel, Germany, in the Microtox® Luminometer Model 500 (AZUR, Environmental, U.S.A.). The EC50 values were estimated using the MicrotoxOmniTM software package.

3.3.7. Chronic toxicity on growth of microalgae *Dunaliela salina* – AlgalTox

The chronic toxicity of the sediment extracts was determined by measuring the *in vivo* growth inhibition of the marine plankton *Dunaliella bioculata* (syn. *D. salina*) due to exposure to model toxic substances and extracts during a three-day period. This is an adapted method for this relevant marine plankton species according to the ISO-certified method for the determination of growth inhibition of algae in the presence of test compounds (ISO/FDIS 8692, 2004). The initial algal density at the beginning of the experiment was determined by measuring the optical algal density (A 680 nm) using a multifunctional microplate reader - spectrophotoflourimeter (Tecan Infinite M200 Pro with the Magellan software). The microalgae were incubated in the presence of the extract samples for 72 hours (3 days) at room temperature. The optical density of the algae was determined at the beginning, daily (during the 3 days) and at the end of the incubation period. The results of growth inhibition of the marine plankton – *D. bioculata* were calculated as the percentage of survival rate, i.e. the growth intensity of the unicellular algae compared to the control group not exposed to the test extracts (negative control) and compared to the cells exposed to a series of concentrations of the modelled toxic compound (positive control).

3.3.8. Inhibition of flax *Linum usitatissimum* seeds germination – Phytotoxicity

For the phytotoxicity test, 4 g of dried sediment were eluted (extracted) for 24 hours (shacking) with ultrapure water (1:10 sediment/water) according to Mamindy-Pajany et al. (2011). The pH values of the eluates range from 7.97 to 8.19 and salinity from 1.21 to 1.77 measured by HI98194 pH/EC/DO Multiparameter (Hanna Instruments, USA) (**Table 3**).

Table 3: Characterisation of marine sediment eluates (water extracts) by Hanna Instruments pH/EC/DO Multiparameter.

Parameters (units)	**Sampling sites**			
	S6	**S7**	**S8**	**S9**
	RV001	**Cuvi-1**	**Cuvi-2**	**Cuvi-3**
pH	7.97	8.13	8.00	8.19
ORP [mV]	267.8	251.2	256.0	247.2
D.O. [%]	50.50	41.90	44.00	35.00
D.O. [ppm]	4.13	3.42	3.66	2.90
EC [µS/cm]	2355	3379	3166	3195
RES [KOhm-cm]	0.4	0.3	0.3	0.3
TDS [ppm]	1181	1690	1584	1599
Salinity [psu]	1.21	1.77	1.65	1.67
Temp. [°C]	24.34	24.46	24.42	24.36

The germination test was performed on flax seeds (*Linum usitatissimum*) at 25°C for a test period of 72h (Pelikan et al., 2022). The flax seeds are purchased commercially (Bio Zone, Croatia). The seeds were sorted according to size and appearance. For each replicate

(3) of the sediment sample, three sheets of filter paper were placed in Petri dishes (100 × 15 mm). Three groups of 30 seeds were placed in each Petri dish (at least 0.5 cm from the edge of the filters), and 5 ml of eluate (equivalent to 100 g/L of sediment d.w.) or ultrapure water added to the filter papers for the sediment samples and controls, respectively. The Petri dishes were covered and placed in a humidified thermostat (HERA Cell 150, Heraeus) in the dark. After 24 hours, the percentage of germination of the seeds was determined.

The percentage of seed germination inhibition (SIG) compared to the test control (deH2O) was calculated as follows:

$$SIG = (Ct – S)/Ct \times 100$$

where Ct is the mean value of seed germination in the controls and S is the mean value of seed germination in the samples.

3.3.9. Probability of Toxic Effects - Risk Posed by Chemical Mixtures

Contaminants rarely occur in sediment as single chemicals. Mean ERM quotients (MERMQs) have been developed to assess the adverse effects of mixtures of chemicals by normalizing the concentration of each substance with respect to its respective ERM value, summing the quotients for each substance, and dividing the sum by the number of chemicals assessed (Long et al., 2000; 2006).

Probability of toxicity has been determined in relation to MERMQs for mixtures of chemicals in the US using amphipod bioassays; however, this association cannot be exported to other areas with different benthic assemblages and sedimentary condition and cannot be used to determine sediment toxicity by using metals alone. The method has been refined by Fairey et al. (2001) who, after assessing 18 methods of calculating MERMQ, determined that using nine of the most predictive SQGs resulted in a closer relationship between toxicity and sediment chemistry. To estimate the probability of a toxic effect, logistic regression models were applied to the concentration data of sediment contaminants, as proposed by Field et al. (2002). The authors described individual logistic regression models for 37 chemicals of potential concern in contaminated sediments that link sediment chemistry to the probability of toxic effects in standard 10-day survival tests for the marine benthic amphipods *Ampelisca abdita* and *Rhepoxynius abronius*.
The equation shows the calculation of the probability of toxic effects:

$$P = [\exp(B0 + Bi\ (x))]/[1+\exp(B0 + Bi\ (x))]$$

where P is the probability of observing a toxic effect, B0 is the intercept parameter, Bi is the slope parameter and x is the chemical concentration or the logarithmic chemical concentration.

The logistic regression models for individual chemicals were combined to obtain a single probability of toxic effect using two approaches: (1) the maximum probability model (Pmax), which was derived from the single chemical model with the highest probability for a sample, and (2) the average probability model (Pavg), which was derived from the arithmetic mean of the probabilities from models for all chemicals measured for a sample. The relationship between the maximum (or mean) toxicity probability of each chemical model and the proportion of toxic samples was described by a binomial least-squares regression model of the interval data. The binomial models were used to estimate the probability of toxicity for individual samples.

3.3.10 Evaluation of Sediment Contamination

In Europe, regulation of contaminated sediments is less coherent, with individual member states developing sediment quality guidelines (SQGs) and monitoring strategies largely independently. However, sediment quality assessment is still subject to a number of uncertainties and insufficient information in terms of regulation, analytical methods, risk assessment and risk management. In general, international regulations have been translated into a national guideline directive for coastal dredged material management and marine sediment quality. Contamination of marine sediments leading to toxic effects is a problem worldwide, especially in countries with a long industrial history.

3.3.10.1 French SQGs

The French sediment quality guidelines (SQGs) are using two guide levels (N1 and N2) for the concentrations of selected metals (As, Cd, Cu, Ni, Pb, Zn, Hg and Cr), ΣPAHs, and ΣPCBs in the total sediment (Mamindy-Pajany et al., 2011; Alzieu and Quiniou, 2001). Total PAHs are defined by the sum of 16 PAHs (Naphtalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Dibenzo(ah)anthracene, Benzo(ghi)perylene and Indeno(1,2,3,c,d) pyrene). The total PCBs are expressed as Aroclor 1260. Further the calculated ∑QN1 and QPECm risk quotients are used as appropriate indicators of potential ecological effects on the aquatic environment under study. ∑QN1 represents the sum of all individual ratios between the contaminant concentration and the French N1 legal level (PEC value). The general sediment risk quotient QPECm represents the averaged ∑QN1 value for a given sediment divided by the number of all pollutants studied, taken from the French guidelines for the management of marine dredged sediment (Alzieu & Quiniou, 2001), as presented in Equation (1).

$$QPECm = (\Sigma ni=1\ (Ci/PECi))/n \quad (1)$$

where Ci is the measured contaminant concentration, PECi is the predicted effect concentration, and n is the number of measured contaminants (n = 10).

Thus, the risk quotient (QPECm) is less than 1 when the contaminant concentration is less than the N1 level, and greater than 1 when the contaminant concentration exceeds N1.

3.3.10.2 Croatian SQGs

The present study, which was only partially carried out as part of the areal, temporal and methodological time investigations, used Croatian national guidelines (MFSD) and threshold values on sediment quality according Bakke et al. 2010. Where the classification of pollutants in sediments is based on the pollutant concentrations (inorganic, organic compounds and emerging), and categorisation according contaminants values (mg/kg d.w.) from: I Background, II Good, III Moderate, IV Bad and V Very bad sediment category.

3.3.11 Data analyses

The calculation of sediments Water – TOC content, Grain size analysis, Probability of Toxic Effects, French sediment quality ∑QN1 and QPECm risk quotients, acute and chronic toxicity, and phytotoxicity were calculated using MS Office - Excel (Microsoft, USA). The correlation among chemical analyses and toxicity results of investigated sites were performed using STATISTICA 8.0 (StatSoft Inc, U.S.A.).

4. RESULTS

4.1 SEDIMENT CHARACTERISATION

Sediment quality is very important in shaping the structure and function of marine environment, especially for benthos community. Grain-size data of the aquatic sediments are of prime importance in differentiating various depositional microenvironments and are also useful for assessing the textural characteristics of the habitat, investigated sites in ecological, toxicological studies and GES monitoring.

4.1.1 Sediment grain size, Water content and TOC

The control sample RV001 is dominated by sand (62.48%) and mud (33.84%); however, it contains less percentage of mud compared to S7 – S9 sediments collected at transect in vicinity of UPOV Cuvi outlet (**Figure 4**). Sample S6 is therefore classified as muddy sand with a mean size of < 0.125 µm. Sediment samples S6 – RV001 is typical eastern Adriatic coarse-grained sediments in which sand fraction dominates (Fütterer & Paul, 1976; Vdović & Juračić, 1993; Pikelj et al., 2009; Pikelj, 2010; Pikelj et al., 2016).

Sediment samples CUVI-1, CUVI-2 and CUVI-3 are the finest sediments from the analysed set with mud (silt & clay content) as the dominant fraction (S7 64.33%, S8 54.20% and S9 68.88%) and higher TOC content, respectively (**Table 4**).

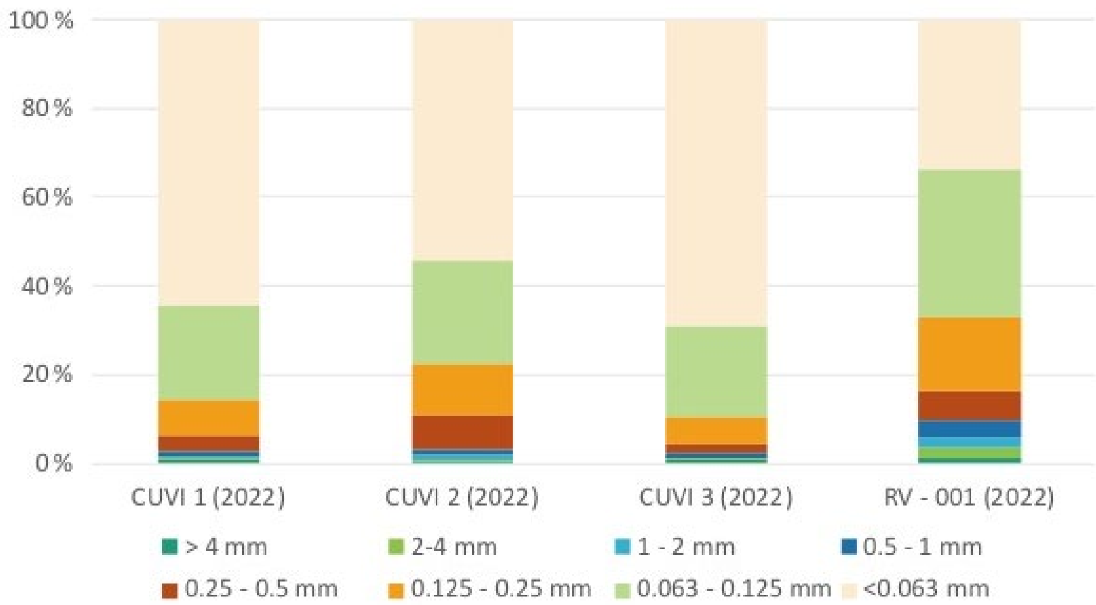

Figure 4: Results of investigated sediments grain size analysis.

Table 4: Grain size composition of investigated sediments with water and total organic carbon content.

Granulometry	Location	S6 RV001	S7 CUVI-1	S8 CUVI-2	S9 CUVI-3
Gravel	Fraction 4 mm (%)	1.23	0.64	0.27	0.67
	Fraction 2 mm (%)	2.45	0.45	0.46	0.32
Sand	Fraction 1 mm (%)	2.21	0.66	1.25	0.33
	Fraction 500 µm (%)	3.86	1.12	1.25	0.87
	Fraction 250 µm (%)	6.40	3.23	7.76	2.26
	Fraction 125 µm (%)	16.73	8.17	11.33	5.93
	Fraction 63 µm (%)	33.28	21.41	23.48	20.74
Mud	Silt and clay (%)	33.84	64.33	54.20	68.88
Total dry weight (%)		100	100	100	100
	Total gravel (%)	3.68	1.08	0.73	0.99
	Total sand (%)	62.48	34.59	45.07	30.13
	Total mud (%)	33.84	64.33	54.20	68.88
Water content (%)		**28.99**	**35.87**	**39.86**	**39.31**
TOC (%)		**0.96**	**1.79**	**1.94**	**1.59**

4.2 CHEMICAL CONTAMINANTS LOAD

4.2.1 PAHs

The results of this study indicate a relatively low concentration of PAHs in marine sediments collected at control site RV001 (∑PAHs 68.5 µg/kg d.w.) as well as at sites in the vicinity of UPOV Cuvi (∑PAHs Cuvi-1 66.8; Cuvi-2 41.4; Cuvi-3 41.3 µg/kg d.w.) (**Table 5**).

Table 5: Concentrations of 16PAHs with ERL/ERM values and PAH ratios in sediments of investigated area.

PAHs		Sampling sites				ERL	ERM
(µg/kg d.w.)	(rings)	S6 RV001	S7 Cuvi-1	S8 Cuvi-2	S9 Cuvi-3		
Naphtalene	2	<1.0	<1.0	<1.0	<1.0	160	
Acenaphthylene	3	<5.0	<5.0	<5.0	<5.0	44	
Acenaphthene	3	<1.0	<1.0	<1.0	<1.0	19	
Fluorene	3	1.0	1.0	1.0	1.0	19	540
Phenanthrene (Phe)	3	4.9	7.4	5.4	3.8	240	1,5
Anthracene (Ant)	3	1.0	1.2	1.0	1.0	85	1,1
Fluoranthene (Flt)	4	10.1	12.4	5.9	6.2	600	5,1
Pyrene (Pyr)	4	9.5	12.0	5.4	5.6	665	2,6
Benzo(a)anthracene	4	4.8	4.8	2.5	2.5	261	1,6
Chrysene	4	4.4	3.6	2.2	2.2	384	2,8
Benzo(b)fluoranthene	5	12.9	5.7	5.7	7.2	320	1,88
Benzo(k)fluoranthene	5	4.6	4.1	2.7	2.5	280	1,62
Benzo(a)pyrene	5	6.3	6.0	3.6	3.2	430	1,6
Dibenzo(a,h)anthracene	5	1.0	1.0	1.0	1.0	63	260
Benzo(g,h,i)perylene	6	5.3	4.8	3.2	3.2	85	1,6
Indeno(1,2,3-c,d)pyrene	6	2.7	2.8	1.8	1.9	240	950
∑PAHs	2-6	**68.5**	**66.8**	**41.4**	**41.3**	**3,672**	**23,150**
Ant/(Ant+Phe) [1,2]	3	0.17	0.14	0.16	0.21	-	-
Flt/(Flt+Pyr) [1,2]	4	0.52	0.51	0.52	0.53	-	-
Phe/Ant [3]	3	4.90	6.17	5.40	3.80	-	-
Flt/Pyr [3]	4	1.06	1.03	1.09	1.11	-	-

[1] Ratios of Ant/(Ant+Phe) < 0.1 and Flt/(Flt+Pyr) < 0.4 indicate petrogenic sources,
[2] Ratios of Ant/(Ant+Phe) > 0.1 and Flt/(Flt+Pyr) ≥ 0.4–0.5 indicate pyrogenic sources,
[3] Ratios of Phe/Ant > 15 and Flt/Pyr < 1 indicate a dominance of petrogenic sources.

The distribution patterns of PAHs (3 to 6-rings) shown in indicate that 4- and 5-ring PAHs dominate (70% at all sampling sites), consistent with pyrogenic origin (Table 5). The enrichment of 4-ring PAHs, particularly fluoranthene and pyrene, indicates greater combustion of diesel fuel. The ratios of Phe/Ant and Flt/Pyr have long been used to distinguish pyrogenic sources from petrogenic ones Yunker et al., 2002). Ratios of Phe/Ant > 15 and Flt/Pyr < 1 indicate a dominance of petrogenic sources (Zhang et al., 2005; Kim et al., 2009). In addition, ratios of Ant/(Ant+Phe) < 0.1 and Flt/(Flt+Pyr) < 0.4 indicate petrogenic sources and ratios of Ant/(Ant+Phe) > 0.1 and Flt/(Flt+Pyr) ≥ 0.4–0.5 indicate pyrogenic sources (Hwang et al., 2003). A higher Flt/(Flt+Pyr) ratio > 0.5 has been used for grass, wood and coal combustion sources (Fadzil et al., 2008; Kumar et al., 2014).

4.2.2 Trace metals

Comparing obtained results (**Table 6**) from the studied sites in Rovinj with the trace metal concentrations in surface sediments at most stations (National Monitoring Programme Projekt Jadran results) along the eastern Adriatic coast, it can be concluded with some exceptions that the Croatian coastal area is not significantly contaminated with trace metals (Institut za oceanografiju i ribarstvo, 2005).

Table 6: Results of sediments Trace metal and PCBs analyses.

Parameters (units)	**Sampling sites**			
	S6 RV001	**S7 Cuvi-1**	**S8 Cuvi-2**	**S9 Cuvi-3**
As / (mg/kg d.w.)	4.10	7.10	6.50	5.00
Cd / (mg/kg d.w.)	0.05	0.12	0.09	0.07
Cu / (mg/kg d.w.)	6.5	15,00	13.00	10.00
Ni / (mg/kg d.w.)	8.4	20,00	17.00	17.00
Pb / (mg/kg d.w.)	9.00	21.00	13.00	13.00
Zn / (mg/kg d.w.)	34.00	66.00	49.00	45.00
Hg / (mg/kg d.w.)	0.06	0.20	0.10	0.10
Cr / (mg/kg d.w.)	19.00	30.00	29.00	28.00
ΣPCBs / (mg/kg d.w.)	0.10	0.10	0.10	0.10

4.2.3 PCBs

The uniform concentrations of PCBs (<0.10 mg/kg d.w.) measured in surface sediments in this study (**Table 6**) were lower or in the range of those previously found in the unpolluted Rovinj costal area 0.01 – 0.278 mg/kg d.w. (Pelikan et al., 2022), reflecting restrictions on the use and production of these compounds. The local effects of UPOV Cuvi wastewater treatment activities didn’t resulted in higher PCB concentration in sediment samples (S7 – S9) in the vicinity of outlet.

4.3 POTENTIAL TOXICITY ASSESSMENT ACCORDING US EPA

Calculated probability of toxicity of investigated sediments on the basis of sediment chemical analyses results content are low for all UPOV Cuvi sites (Pavg 0.19-0.22; Pmax 0.21-0.23), but higher than for control RV001 site (Pavg 0.18; Pmax 0.15) (**Table 7**).

Table 7: Results of contamination evaluation by calculation of probability of toxic effects (Pavg, Pmax) on the basis of sediment chemical analyses.

Parameters (units)	**Sampling sites**			
	S6	**S7**	**S8**	**S9**
	RV001	**Cuvi-1**	**Cuvi-2**	**Cuvi-3**
P_{avg}	0.18	0.22	0.19	0.19
P_{max}	0.15	0.23	0.21	0.21

4.4 TOXICITY OF SEDIMENT EXTRACTS

4.4.1. Acute toxicity

Toxic potential values for sediment samples expressed as EC50 (estimated concentration to reduce the bacterial luminescence for 50%) are shown in **Table 8**. EC50 values had an ascending order as the lowest EC50 score was the most toxic while the highest EC50 score was the least toxic.

Table 8: Results of sediment acute toxicity determined by inhibition of bacterial light emissions in Microtox test expressed as EC50 values with CI confidence interval (95%).

Parameters (units) Acute toxicity Microtox	**Sampling sites**			
	S6	**S7**	**S8**	**S9**
	RV001	**Cuvi-1**	**Cuvi-2**	**Cuvi-3**
EC50 (mg)	97	48	22	30
Confidence interval	53-150	22-65	11-33	17-45

Toxic potential values of sediment extracts were in the range of 22-97 mg. Reference site RV001 had a EC50 value of 97 mg and was the least toxic comparing to UPOV Cuvi sites Cuvi-1 (48 mg), Cuvi-2 (22 mg) and Cuvi-3 (30 mg).

4.4.2. Chronic toxicity

The chronic toxicity of the tested samples is expressed as a percentage (%) of cell growth (AlgalTox) compared to untreated algal cells that were not exposed to sediment extracts, i.e. cells that were exposed only to the solvent (negative control - DMSO) (**Table 9**). Seawater extracts of sediment samples in the vicinity of UPOV Cuvi (samples S7-S9) did not show a difference in the toxic response compared to the samples collected at three control locations (RV001). The growth of algae exposed to increasing concentrations of sediment extracts collected in the area of the UPOV Cuvi were practically the same as in the case of algae exposure to sediment extracts that were collected at control location. Conversely, algae exposed to the model toxic compound potassium bichromate ($K_2Cr_2O_7$; 10 mg/L) showed approximately 50% growth inhibition, as expected.

Table 9: Results of sediment chronic toxicity determined by measuring sediment extract inhibition of microalgae *D. salina* growth (AlgalTox) expressed as percentage of cell growth (mean ± SD).

Parameters (units) Chronic toxicity	**Sampling sites**			
	S6	**S7**	**S8**	**S9**
	RV001	**Cuvi-1**	**Cuvi-2**	**Cuvi-3**
AlgalTox (%)	93.33 ± 4.87	87.14 ± 4.37	85.90 ± 5.29	94.15 ± 4.66
Negative control (DMSO)	94.05 ± 3.79	**Pozitive control ($K_2Cr_2O_7$; 10 mg/L)**		52.19 ± 1.40

The chronic toxicity test of sediment extract using the marine phytoplankton *D. salina* does not differentiate sediments of the control area RV001 from sediments of UPOV

Cuvi (S7 – S9) sites. Furthermore, the obtained EC50 values (>100 mg of dry sediment per mL of algae exposure medium) clearly indicate a good environmental status of the marine sediments in the Rovinj coastal area.

4.4.3. Phytotoxicity

The phytotoxicity assay conducted with seeds of flax *Linum usitatissimum* showed inhibition of germination with 5 mL of sediment eluates (equivalent to 100 g/L sediment d.w.) with increasing effect: S6 < S9 < S8 < S7 (**Table 10**). In general, there is clear growing phytotoxicity (SIG) effect of sediments in vicinity of UPOV Cuvi. As a test control we used deH_2O as the reference value for inhibition (0%). Finally, PI allows the discrimination of two groups: S6 (3.60%), S9 (12.90%) and S8 (14.12%) as less affected sediments compared to the moderate phytotoxic sediments S7 (23.50%).

Table 10: Results of sediment average phytotoxicity determined by seed germination test (SIG) using flax *L. usitatissimum*.

Parameters (units)	**Sampling sites**			
	S6	**S7**	**S8**	**S9**
	RV001	**Cuvi-1**	**Cuvi-2**	**Cuvi-3**
SIG (%)	3.60	23.50	14.12	12.90
StDev	2.29	3.38	3.94	5.03

4.5 EVALUATION OF SEDIMENT CONTAMINATION USING SQGS

Table 11: Summary results of physico-chemical and ecotoxicological analyses of sediment samples from reference site RV001 (S6) and transect sites near UPOV Cuvi (S7-S9).

Parameters (units)	Sampling sites			
	S6	S7	S8	S9
	RV001	Cuvi-1	Cuvi-2	Cuvi-3
As / (mg/kg d.w.)	4.10	7.10	6.50	5.00
Cd / (mg/kg d.w.)	0.05	0.12	0.09	0.07
Cu / (mg/kg d.w.)	6.50	15.00	13.00	10.00
Ni / (mg/kg d.w.)	8.40	20.00	17.00	17.00
Pb / (mg/kg d.w.)	9.00	21.00	13.00	13.00
Zn / (mg/kg d.w.)	34.00	66.00	49.00	45.00
Hg / (mg/kg d.w.)	0.06	0.20	0.10	0.10
Cr / (mg/kg d.w.)	19.00	30.00	29.00	28.00
ΣPAHs / (mg/kg d.w.)	0.07	0.07	0.04	0.04
ΣPCBs / (mg/kg d.w.)	0.10	0.10	0.10	0.10
ΣQ_{N1}	1.40	2.78	2.19	2.02
Q_{PECm}	0.14	0.28	0.22	0.20
P_{avg}	0.18	0.22	0.19	0.19
P_{max}	0.15	0.23	0.21	0.21
Microtox EC50 (mg)	97	48	22	30
AlgalTox (%)	93.33	87.14	85.90	94.15
Phytotoxicity (%)	3.60	23.50	14.12	12.90

Table 12: Overview of SQGs threshold values used for evaluation of sediment contamination in this study.

Parameters (units)	Croatia Bakke et al. (2010)	ERL – Effect of low range	ERM – Effect of median range	France	
	I Category Background levels	10 percentile	50 percentile	N1 Legal level	N2 Legal level
As / (mg/kg d.w.)	<20	8.20	70.00	25	50
Cd / (mg/kg d.w.)	<0.25	1.20	9.60	1.2	2.4
Cu / (mg/kg d.w.)	<35	34.00	270.00	45	90
Ni / (mg/kg d.w.)	<30	20.90	51.60	37	74
Pb / (mg/kg d.w.)	<30	46.70	218.00	100	200
Zn / (mg/kg d.w.)	150	150.00	410.00	276	552
Hg / (mg/kg d.w.)	<0.15	0.15	0.71	0.4	0.8
Cr / (mg/kg d.w.)	<70	81.00	370.00	90	180
ΣPAHs / (mg/kg d.w.)	<0.300	4.022	44.792	1.5	15
ΣPCBs / (mg/kg d.w.)	<0.005	22.70	180,00	0.5	1.0
ΣQ_{N1}	-	-	-	-	-
Q_{PECm}	-	-	-	-	-
P_{avg}	-	-	-	-	-
P_{max}	-	-	-	-	-
Microtox (EC50)	-	-	-		
AlgalTox (LT50)	-	-	-		
Phytotoxicity (%)	-	-	-	-	-

With the goal of ecological risk assessment, specific numeric sediment quality guidelines have been developed to evaluate the adverse biological effects of various contaminants. The international SQGs for PAHs, PCBs an TMs were derived from the results of numerous laboratory and field studies and various toxicity tests for different aquatic organisms (Long et al., 1996; Macdonald et al., 2000). The lower effect range (ERL) and the median effect range (ERM) have been used to evaluate potential adverse toxicological effects (Long et al., 1995). These ranges are used to define concentrations of contaminants that are rarely (<ERL), occasionally (≥ERL and <ERM) or frequently (≥ERM) associated with adverse biological effects.

In this study, total concentrations and individual concentrations of PAHs, total PCBs and TMs were compared with national SQS, ERL and ERM threshold values, as well as France SQS N1 and N2 contaminant legal level (**Table 10 and 11**). The results showed that the total concentrations of contaminant at all S6-S9 sites were significantly lower than the ERL and ERM values. This means that the contaminant concentrations in the sediments of the studied area sites, provided by chemical analyses are in a range where no harmful toxicological effects are expected.

Table 13: Correlation coefficients (r) of metals (As, Cd, Cu, Ni, Pb, Zn, Hg, Cr), ΣPAHs, ΣPCBs, ΣQN1 and QPECm evaluation, probabilities of a toxic effect (Pavg and Pmax), and toxicity results of marine sediments contamination analyses (Marked values (black font, bolded) are significant at $p < 0.05$, where the grey-filled cells indicate positive and blue-filled cells a negative correlation).

Variables	As	Cd	Cu	Ni	Pb	Zn	Hg	Cr	ΣPAHs	ΣQ_{N1}	Q_{PECm}	P_{avg}	P_{max}	Microtox	AlgalTox	Phytotoxicity
As		**0.9702**	**0.9909**	0.8652	0.8544	0.9248	0.8360	0.8560	-0.0631	0.9414	-0.7649	0.8206	0.8626	-0.6245	-0.8942	-0.7036
Cd	**0.9702**		**0.9754**	0.8745	**0.9537**	**0.9878**	0.9439	0.8263	0.0967	**0.9817**	-0.7251	0.9345	0.8701	-0.5185	-0.7900	-0.6476
Cu	**0.9909**	**0.9754**		0.9241	0.8847	0.9468	0.8624	0.9100	-0.1169	0.9716	-0.8318	0.8440	0.9219	-0.6823	-0.8295	-0.7735
Ni	0.8652	0.8745	0.9241		0.8470	0.8889	0.8120	**0.9861**	-0.3231	0.9441	**-0.9590**	0.7846	**0.9999**	-0.8206	-0.5770	-0.9218
Pb	0.8544	**0.9537**	0.8847	0.8470		**0.9875**	**0.9980**	0.7582	0.2294	0.9624	-0.6618	**0.9941**	0.8412	-0.3957	-0.5773	-0.5748
Zn	0.9248	**0.9878**	0.9468	0.8889	**0.9875**		**0.9793**	0.8225	0.1304	**0.9894**	-0.7275	**0.9710**	0.8841	-0.4929	-0.6848	-0.6469
Hg	0.8360	0.9439	0.8624	0.8120	**0.9980**	**0.9793**		0.7161	0.2900	0.9453	-0.6134	**0.9990**	0.8056	-0.3377	-0.5658	-0.5223
Cr	0.8560	0.8263	0.9100	**0.9861**	0.7582	0.8225	0.7161		-0.4558	0.8965	**-0.9868**	0.6838	**0.9877**	-0.8987	-0.6093	**-0.9643**
ΣPAHs	-0.0631	0.0967	-0.1169	-0.3231	0.2294	0.1304	0.2900	-0.4558		-0.0153	0.5779	0.3333	-0.3333	0.7981	0.0288	0.6645
ΣQ_{N1}	0.9414	**0.9817**	**0.9716**	0.9441	**0.9624**	**0.9894**	0.9453	0.8965	-0.0153		-0.8187	0.9305	0.9407	-0.6142	-0.6935	-0.7502
Q_{PECm}	-0.7649	-0.7251	-0.8318	**-0.9590**	-0.6618	-0.7275	-0.6134	**-0.9868**	0.5779	-0.8187		-0.5768	**-0.9621**	0.9465	0.5061	**0.9938**
P_{avg}	0.8206	0.9345	0.8440	0.7846	**0.9941**	**0.9710**	**0.9990**	0.6838	0.3333	0.9305	-0.5768		0.7778	-0.2946	-0.5560	-0.4829
P_{max}	0.8626	0.8701	0.9219	**0.9999**	0.8412	0.8841	0.8056	**0.9877**	-0.3333	0.9407	**-0.9621**	0.7778		-0.8267	-0.5752	-0.9259
Microtox	-0.6245	-0.5185	-0.6823	-0.8206	-0.3957	-0.4929	-0.3377	-0.8987	0.7981	-0.6142	0.9465	-0.2946	-0.8267		0.4573	**0.9735**
AlgalTox	-0.8942	-0.7900	-0.8295	-0.5770	-0.5773	-0.6848	-0.5658	-0.6093	0.0288	-0.6935	0.5061	-0.5560	-0.5752	0.4573		0.4580
Phytotoxicity	-0.7036	-0.6476	-0.7735	-0.9218	-0.5748	-0.6469	-0.5223	**-0.9643**	0.6645	-0.7502	**0.9938**	-0.4829	-0.9259	**0.9735**	0.4580	

5. DISCUSSION

5.1 SEDIMENT GRAIN SIZE AND TOC

Grain size analysis of the investigated sediments shows the dominance of fine fraction in sediments in the coastal area. Control sample RV001 is classified as muddy sand sediment type with a mean size of <0.125 μm which represent typical eastern Adriatic coarse-grained sediments where sand fraction dominates (Fütterer & Paul, 1976; Vdović & Juračić, 1993; Pikelj et al., 2009; Pikelj, 2010; Pikelj et al., 2016). In contrast sediment samples in vicinity of UPOV Cuvi outlet were classified as muddy sediment with silt & clay content as the dominant fraction (**Table 4**). From literature it is reasonable to assume that coarse grain sediments near the coastline have lower organic carbon, but in our case the TOC content at sites in vicinity of UPOV Cuvi are slightly higher (1.59% - 1.79%) vs reference site RV001 (0.96%) probably because of local waste treatment plant outlet input (Herut and Sandler, 2006).

5.2 PAHs

PAHs are toxic compounds and can have harmful biological effects. The results of this study indicate a relatively low concentration of PAHs in marine sediments collected at control site RV001 as well as at sites in the vicinity of UPOV Cuvi (**Table 5**). Comparing results from this study with previous results of Rovinj area (sampling 2011), the PAHs load at all investigated sites are in the range of control - reference area e.g. Lim Bay middle 28.2 μg/kg d.w. and open sea 3NM off Rovinj 103.0 μg/kg (Bihari et al., 2006; Pelikan et al. 2022).

Bihari et al. (2006; 2007) reported total concentrations of PAHs in marine sediments in the Rovinj and Rijeka area, ranging from 213.0 to 695.0 μg/kg d.w., while other studies such as Traven et al. (2008) and Traven (2013) reported that the total concentration of PAHs in local sediment samples collected from the Kvarner Bay ranged from 113.8 μg/kg d.w. for a recreational area to 11,479.0 μg/kg d.w. for an industrial site. The author of (Bajt, 2022) reported that in recent years the situation according to PAH levels in the Gulf of Trieste (Slovenia) falls within the concentration from 100 to 1000 ng/g (moderately polluted areas) with a reducing trend.

Some studies have shown that PAHs have a higher affinity for the fine-grained sediments in various environments, inside and outside of the Adriatic Sea (Magi et al., 2002; Vane et al., 2020). However, the results of this study showed that there is no connection between PAH concentrations found in sediment samples of different textures (e.g., S6 and S9; Table 2). These results suggest that grain size does not play the main role in the distribution of PAHs in studied sediment.

The distribution patterns of PAHs (3 to 6-rings) shown in indicate that 4- and 5-ring PAHs dominate at all sampling sites. The PAH ratios can also be used to identify sources of contamination (Bajt, 2022). The ratios of Phe/Ant and Flt/Pyr have long been used to distinguish pyrogenic sources from petrogenic ones (Yunker et al., 2002). Ratios of Phe/Ant > 15 and Flt/Pyr < 1 indicate a dominance of petrogenic sources (Zhang et al., 2005; Kim et al., 2009). In addition, ratios of Ant/(Ant+Phe) < 0.1 and Flt/(Flt+Pyr) < 0.4 indicate petrogenic sources and ratios of Ant/(Ant+Phe) > 0.1 and Flt/(Flt+Pyr) ≥ 0.4–0.5 indicate pyrogenic sources (Hwang et al., 2003). A higher Flt/(Flt+Pyr) ratio > 0.5 has been used for grass, wood and coal combustion sources (Fadzil et al., 2008; Kumar et al., 2014).

For all samples, it is not surprising that PAHs of pyrolytic origin are prevalent in the region due to the increased traffic of small and large motorboats. This finding is confirmed by a large body of published data indicating that pyrolytic sources dominate PAH contamination of marine sediments of the Mediterranean basin (Notar et al., 2001; Frignani et al., 2003; Bihari et al., 2006; 2007; Cardellicchio et al., 2007; Alebić-Juretić, 2011; Pelikan et al., 2022).

5.3 HEAVY METALS

The concentrations of TMs found in the sediments analyzed for this study correspond to the natural metal content of the Adriatic (Martinčić et al., 1989; Ferrara & Maserti, 1992; Ujević et al., 1998; Bogner et al., 2005; Mikac et al., 2006), and are within the range of values characteristic for pristine to unpolluted areas of the Mediterranean (UNEP, 1996; UNEP, FAO, WHO, 1989; UNEP 1994; UNEP, 1996). For the purpose of analysing metal contamination of sediments, the authors (Obhodaš et al., 2006) divided the sites into seven categories: 1—bays, 2—beaches, 3—villages, 4—ports, 5—marinas-pier areas, 6—marina service areas and 7—others (sea mud, river tributaries etc.). The concentration values of Category 1 sediment samples can be used in defining "normal" or ''natural" background values for concentrations of chemical elements in coastal sediments. Furthermore, Orescanin et al. (2001) and Valković et al. (2007) provides descriptive statistics for the elements Cu, Zn, As and Pb for six categories of coastal sediment samples. Concentration values for all four elements show the same trend: they increase from bays and beaches to villages, ports, marina-piers and marina-service areas. In fact, the high concentrations measured at pollution sources decrease rapidly along the transport pathways of suspended matter (Mandić and Pavela Vrančić, 2017). Concentrations typically decrease by several orders of magnitude as little as 100 m from the pollution source. The full implementation of municipal wastewater collection in the city of Rovinj in 2022 and UPOV Cuvi treatment (2023) are significantly reducing the input of pollution into the local marine environment.

Comparing obtained results (**Table 2**) from the studied sites in Rovinj with the trace metal concentrations in surface sediments at most stations (National Monitoring Programme Projekt Jadran results) along the eastern Adriatic coast, it can be concluded with some exceptions that the Croatian coastal area is not significantly contaminated with trace metals (Institut za oceanografiju i ribarstvo, 2005). Furthermore, the results of the Projekt Jadran have shown that sediment contamination is limited to very narrow coastal areas near urban pollution sources of pollution, so called "hot spots", such as mercury (Hg) in the Kaštela Bay at the Inavinil station and lead (Pb) at the Vranjic station (Institut za oceanografiju i ribarstvo, 2005).

5.4 PCBs

PCBs are synthetic compounds that have been produced since the 1930s. In the Adriatic Sea, whose water renewal period is less than 10 years, there may have been an accumulation of residues of chlorinated compounds that were discharged through the atmosphere.

The PCB concentrations of <0.10 mg/kg d.w. measured in surface sediments in this study (**Table 2**) were lower than those previously found in the Rovinj costal area 0.01 – 0.278 mg/kg d.w. (Pelikan et al., 2022), northern and central Adriatic: 0.9–14.7 (Fowler et al., 2000) or 3–80 mg/kg d.w. (Caricchia et al., 1993) and in the eastern Adriatic: <0.5–29.4 mg/kg d.w. (Picer et al., 1991), reflecting restrictions on the use and production of these compounds. This is consistent with studies showing a significant decrease in PCB concentrations in various environmental compartments of the Mediterranean and Adriatic Seas over the last two decades (Picer, 2000; Combi et al., 2016).

Regarding the relationship between grain size and PCBs, several studies have shown that PCBs preferentially accumulate in the fine-grained fraction of the sediment, similar to other pollutants (e.g., heavy metals, PAHs) and organic matter (TOC) (Pierrard et al., 1996; Zhao et al., 2010). Therefore, one would expect a higher ΣPCBs concentration in Cuvi sediments. However, the local effects of UPOV Cuvi wastewater treatment activities didn't resulted in higher PCB concentration in sediment samples (S7 – S9) in the vicinity of outlet.

5.5 POTENTIAL TOXICITY ASSESSMENT ACCORDING US EPA

Calculated probability of toxicity of investigated sediments on the basis of sediment chemical analyses results are low for all UPOV Cuvi sites (Pavg 0.19-0.22; Pmax 0.21-0.23), but higher than for control RV001 control site RV001 (Pavg 0.18; Pmax 0.15). The results of Pavg and Pmax of this study (**Table 7**) were in range of those previously published for Rovinj costal area unpolluted sites: Lim Bay (0.24; 0.35) and open sea (0.18; 0.15) (Pelikan et al., 2022).

5.6 TOXICITY

Acute toxicity of sediment extracts determined by Microtox test were in the range of unpolluted sites. Reference site RV001 had a EC50 value of 97 mg and was the least toxic comparing to UPOV Cuvi sites Cuvi-1 (48 mg), Cuvi-2 (22 mg) and Cuvi-3 (30 mg). Decreasing EC50 values of sediment at UPOV Cuvi transect (50, 200 and 1000 m) doesn't follow increasing distance from the waste treatment plant outlet (Fafanđel et al., 2015).

The chronic toxicity test (AlgalTox) of sediment extract using the marine phytoplankton *D. salina* does not differentiate sediments of the control area RV001 from sediments of UPOV Cuvi (S7 – S9) sites. Furthermore, the obtained EC50 values (>100 mg of dry sediment per mL of algae exposure medium) clearly indicate a good environmental status of the marine sediments in the Rovinj coastal area.

The phytotoxicity assay conducted with seeds of flax *L. usitatissimum* showed inhibition of germination with of sediment eluates with increasing effect: S6 < S9 < S8 < S7. In general, there is clear growing phytotoxicity (SIG) effect of sediments in vicinity of UPOV Cuvi. Finally, obtained phytotoxicity values allows the discrimination of two groups: S6 (3.60%), S9 (12.90%) and S8 (14.12%) as less affected sediments compared to the moderate phytotoxic sediments S7 (23.50%).

5.7 EVALUATION OF SEDIMENT CONTAMINATION USING SQGS

The concentrations of the measured values of trace metals, individual and total PAHs and total PCBs are below the threshold values ERL (Effect of low range 10 percentile) and TEL (Threshold effect level - rarely) (Long, 1996; MacDonald, 2000), as and the Norwegian SQGs regulation (Bakke et al., 2010), which was accepted by the Republic of Croatia (NN 28/2021). All investigated sediments belong to the first category of marine sediments, which represent a good state of the marine environment, that is, the measured concentrations of potential pollutants were at the level of natural (background) values. Ecological risk assessment and probability of toxic effect determined on the basis of QPECm (< 1) average risk quotient, indicates that the mentioned sediments represent a good environmental condition and that there is no ecological risk for the environment and organisms according to French regulations.

The Water Framework Directive (WFD 2000/60/EC) provides legislation and opportunities for monitoring and regulating the aquatic environment (MSFD 2008/56/EC, London Protocol, OSPAR Convention and Helsinki Convention). Sediments are an essential, integral, and dynamic component of the aquatic ecosystem. Healthy environments need sediments to support life, while also serving as a sink for many hazardous chemicals. Above a certain level of pollution, this leads to negative impacts such as loss of biodiversity (Usero et al., 2008). Although there is a link between sediment quality and the achievement of good ecological status of European waters, the WFD does not specifically address this. Usually, the strategy for elaborating the guidance values is based on a statistical evaluation of the pollutant concentrations, measured during the multi-annual campaigns. The study of the distribution of the results allows determining the value of “background noise”, i.e., the natural content without any recognizable anthropological contribution. Direct comparison of national action levels/standard guidance values for sediment contaminants between countries is not possible due to the use of different classification systems, grain size fractions (standardization) in which analyses must be performed, chemical parameters (metals, organic contaminants, nutrients, etc.), and ecotoxicity bioassays where applicable. National regulations and standards for metals and organic contaminants refer, for example, to the grain size fraction: <20 µm in Germany, <63 µm in Spain and total dry weight in Denmark, Norway, Ireland, the United Kingdom, France, Belgium, the Netherlands and the Adriatic and Ionian Seas. However, the heterogeneity of monitoring and analysis protocols may limit the comparability of data, although environmental assessment and large geographic extent require consistency. Keeping adequate documentation of monitoring and analysis protocols is essential to improve data comparability.

Recently, a document was produced within the framework of the Interreg ADRION “HarmoNIA methodological proposals” on the Adriatic-Ionian marine sub-regions, by assessment of contamination from hazardous substances (Chemical status of the WFD; Descriptor 8 of the MSFD; Ecological Objective 09 of EcAp/IMAP) and analyses of heterogeneity of monitoring procedure for the Mediterranean Region and Adriatic Sea. The greatest homogeneity between institutions was found in terms of analytical tools and sediment sampling (box corer etc.), but the thickness of the sampled sediment layer varied (surface 10, 5 and 2 cm) as well as the analyses of the different grain size fractions (<63 µm, <0.5 mm, <2 mm, unsieved). Consistent with observed gaps in regional/national harmonisation of SQGs and contaminants threshold values, the authors have undertaken emerging contaminants analyses e.g., microplastics which is in progress.

Even the low contamination load of investigated sites in this study, both for reference site RV001 and sites in vicinity of UPOV Cuvi categorized as I category – unpolluted areas with background levels of PAHs, PCBs and TMs, it is possible to observe low acute/chronic/phytotoxic effect at sites in vicinity of wastewater treatment plant outlet (Silva et al., 2004). Probably, the aquatic environment faces increasing threats from a variety of unregulated organic chemicals originating from human activities, collectively known as chemicals of emerging concern (CECs). These include pharmaceuticals, personal care products, pesticides, surfactants, industrial chemicals, and their transformation products. CECs enter aquatic environments through various sources, including effluents from wastewater treatment plants, industrial facilities, runoff from agricultural and residential areas, as well as accidental spills. Data on the occurrence of CECs in the marine environment are scarce (Shahid et al., 2021), and more information is needed to assess the ecological status of water bodies and prioritize toxic chemicals for further studies or risk assessment. Chemical analyses due to the low concentrations of CECs in the environment and the sampling system, e.g. a device for the extraction of a large amount of samples on site via the solid phase (LVSPE) and the necessary complex equipment with regard to the analysis methodology, are very demanding and are not covered by the existing international regulations (Pastorino and Ginebreda, 2021).

A solution for assessing the good state of the environment, which would include monitoring of classic pollutants (TMs, PAHs, PCBs) and evaluating the possible presence of low concentrations of CECs, is the inclusion of a battery of toxicity tests determining the biological effects of pollutants on local biota. As a continuation of our research previous research of Rovinj costal area – marine sediment contamination, we decided to conduct widely accepted toxicity tests of sediments extracts (MeOH/DCM and water eluates) as an upgrade to previous sediment chemical analyses (Mamindy-Pajany et al., 2011; Duran et al., 2015; Pelikan et al., 2022).

The scientific contribution of this research is the spatial analysis of marine sediment samples of the Rovinj coastal area in accordance with the MFSD in order to determine the good environmental state, i.e. the determination of zero state necessary for monitoring the operation of the UPOV Cuvi municipal wastewater treatment plant in the future.

6. CONCLUSIONS

- The results of this study Physico-Chemical and Ecotoxicological Evaluation of Marine Sediments Quality in vicinity of communal outlet Cuvi represent the first evaluation of UPOV Cuvi (Rovinj Coastal Area, NE Adriatic Sea, Croatia) wastewater treatment plant discharge effects on the local costal marine sediments.
- The sediments in the vicinity of the UPOV Cuvi outlet have slightly higher values of total organic carbon (TOC) with silt-mud fraction predomination (S7, 64%; S8, 54% and S9, 69%). In contrast to them, the sediment from the control location S6 contains only 34% silt-mud.
- Results of chemical (PAHs, PCBs, trace metals) analyses of investigated marine sediments identified as background values, categorized sediments of all sites (Cuvi-1, Cuvi-2 and Cuvi-3) and control site S6 RV001 as unpolluted areas. All pollutants were below the International threshold ERL (Effect of low range 10 percentile) and TEL (Threshold effect level - rarely) values, as well as national threshold values (Official Gazette NN 28/2021).
- By calculation of probability of toxic effect (Pmax; Pavg US EPA) on basis of PAHs, PCBs and trace metals values, it was possible to distinguish studied sites in vicinity of UPOV Cuvi (S7, 2.78; S8, 2.19; S9, 2.02) from control site RV001 (S6, 1.40).
- By applying appropriate French guidelines for sediment quality (ΣQN1) it was possible to distinguish studied sites in vicinity of UPOV Cuvi (S7, 2.78; S8, 2.19; S9, 2.02) from control site RV001 (S6, 1.40), but the general risk analysis with QPECm values < 1.00 (S7 0.28; S8 0.22; S9 0.20) indicated absence of potential ecological impacts to the local marine environment under study.
- In conclusion, there is no evidence of possible negative ecological impact of human activities on the studied marine environment in the coastal area of Rovinj. All investigated sediments belong to the first category of marine sediments and represent a good state of the environment and that there is no ecological risk for the environment and organisms at the locations.
- Sediment quality guidelines (SQGs) are an important tool for assessing pollution of marine sediments. Although such guidelines are not definitive indicators of toxicity (if toxicity tests are not included), they can have highly predictive ability and are a useful tool for identifying areas of potential adverse biological impacts.
- Bioassays do not respond in the same manner as sediment chemistry analyses, suggesting that extraction/bioavailability and the combined effects of contaminants may play a significant role in the observed "true" toxicity effects.
- In order to monitor the marine environment with a special attention to marine sediments and to obtain GES, there is an urgent need to collect data on sediment quality along the eastern Adriatic in terms of pollutant concentrations, including emerging pollutants e.g.
- Even, the sediment samples did not show any evidence of contamination by the wastewater treatment facility, the monitoring should be continued along with the CEC chemicals (Contaminants of Emerging Concern - CECs). Therefore, it is necessary to give priority to the research of CEC chemicals, especially in sediments to assess the risk to the environment and human health.
- One overall challenge, irrespective of the geographical context, is finding an acceptable balance between environmental relevance and regulatory acceptance of sediment toxicity testing, while guaranteeing a robust risk characterization.

7. POVZETEK V SLOVENSKEM JEZIKU

Občinske čistilne naprave za odpadne vode igrajo ključno vlogo pri ohranjanju javnega zdravja in varovanju okolja. Vendar pa imajo tudi potencial vplivati na lokalno morsko okolje, če niso pravilno upravljane. Od leta 2021 do 2023 je bila občinska čistilna naprava UPOV Cuvi obnovljena in nadgrajena s tretjo stopnjo čiščenja odpadnih voda. Po eksperimentalnem delu podvodni iztok ne bo več v obratovanju, vse očiščene vode bodo odvajane na celino preko kanalov in uporabljene za namakanje zelenih površin.

Hipoteze:

1. Vnos UPOV Cuvi vpliva na kemično onesnaženje sedimentov na preučevanih mestih v lokalnem morskem okolju.
2. Koncentracije možnih kemičnih onesnaževal (PAH, PCB, težke kovine) se povečujejo vzdolž preučevanih transektov (50, 200, 1000 m) v bližini iztoka UPOV Cuvi.
3. Koncentracije onesnaževal so pozitivno korelirane z rezultati testov toksičnosti, ki odražajo obremenitev onesnaževal izbranih mest.
4. Analize kemičnih onesnaževal v sedimentih, ocenjene v skladu z nacionalnimi in regionalnimi smernicami za kakovost sedimentov, omogočajo klasifikacijo in oceno dobrega stanja okolja brez ekološkega tveganja za lokalno okolje in organizme.
5. Integracija rezultatov toksičnih testov eluatev/ekstraktov sedimentov z analizami kemičnih onesnaževal v sedimentih bo nadalje pokazala odsotnost ali vpliv iztoka UPOV-Cuvi na lokalno morsko okolje in mest vzorčenja, odvisno od obremenitve s onesnaževali.
6. Potencialna toksičnost sedimentov je odvisna od vrste sedimenta.

Metodologija:

Mesta vzorčenja so izbrana glede na SUO UPOV Cuvi in prejšnje rezultate onesnaženja, ob upoštevanju zgodovine onesnaženj in okoljskih pritiskov. Zbiranje površinskih sedimentov (< 3-5 cm) bo potekalo na štirih mestih vzorčenja s pomočjo Van Veen grabulje na referenčnem mestu (RV001) 1 NM od mesta Rovinj in na mestih 50 m, 200 m in 1000 m (južni transekt) od iztoka UPOV Cuvi.

Vsebnost vode v sedimentu (suha teža) bo določena s segrevanjem 10 g pri 105 °C za 24 ur. Prisotnost skupnega organskega materiala bo ocenjena z izgubo teže po segrevanju pri 450 °C za 5 ur. Vzorci za določanje velikosti delcev bodo ločeni s pomočjo mokrega sitra. Frakcija 65 µm bo sušena v pečici pri 90 °C za 24 ur in frakcionirana skozi serijo 6 sito (specifikacija ASTM E11). Nato bo suho sitrano v intervalih po 1 phi do phi+4 (65 µm) in klasificirano s pomočjo trikotne grafike (Buchanan in Kain, 1971) in GRADISTAT (Blott and Pye, 2001). Sedimenti (50 g mokre teže) bodo ekstrahirani z diklormetan-metanol (2:1), kot je opisano pri Schiewe et al. (1985). Ekstrakti bodo izhlapljeni do suhega in raztopljeni v 100 µl dimetil sulfoksida (DMSO) z širokim razponom nepolarnih do polarnih spojin. Ekstraktne praznine bodo pripravljene s pomočjo iste procedure, vendar brez sedimenta.

PAH bodo analizirani in kvantificirani s strani Učne ustanove za javno zdravje Primorsko-goranske županije, Rijeka, kot je opisano pri Pelikan et al. (2022). Sediment (10 g) bo ekstrahiran s 100 mL spektrografskega cikloheksana (Merck, Darmstadt, Nemčija) v ultrazvočni kopeli 45 minut. Ekstrakt bo izhlapen do suhega z uporabo rotacijske evaporatorja, ostanek raztopljen v 1 mL cikloheksana in frakcija PAH ločena na koloni z 1 g silicijevega gela (Kemika, Zagreb, Hrvaška) in Al2O3 na vrhu. Kolona bo eluirana z 25 mL cikloheksana in skoraj izhlapena do suhega na rotacijski evaporator. Preostala frakcija bo raztopljena v 1 mL destiliranega metanola in analizirana z HPLC (Linšak et al., 2012).

Metali bodo analizirani in kvantificirani s strani Učne ustanove za javno zdravje Primorsko-goranske županije, Rijeka (Linšak et al., 2012). Vzorci sedimentov bodo sušeni čez noč v pečici pri 105 °C. Vzorci (1 g, < 2 mm frakcija velikosti delcev) bodo nato segreti s HNO3 v mikrovalovni pečici (PerkinElmer/Anton Paar Multiwave 3000). Vsebnost kovin bo določena z uporabo Perkin Elmer (Waltham, MA, ZDA) 4110 ZL (Pb, Hg in Cd) in Perkin Elmer Analyser 200 (Cu in Zn). Pb in Cd bosta določena z metodo grafitne peči atomske absorpcijske spektroskopije (GFAAS), medtem ko bo Hg določen z metodo sistemov za injekcijo živega srebra (FIMS). Cu, Zn in drugi metali bodo določeni z direktno plamensko atomizacijo.

PCB bodo analizirani in kvantificirani kot Aroclor 1260 s strani Učne ustanove za javno zdravje Primorsko-goranske županije, Rijeka (Pelikan et al., 2022). Približno 20 g mokrega sedimenta bo homogeniziran z anhidridnim NaSO4 in ekstrahiran z n-hekzanom (Linšak et al., 2012). Ekstrakti bodo analizirani z GC/MS–QP2010 (Shimadzu Corp., Kyoto, Japonska) opremljenim z AOC-5000 avtoinjektorjem (CTC Analytics AG, Twingen, Švica).

Za oceno verjetnosti toksičnega učinka bodo uporabljeni logistični regresijski modeli na podatkih o koncentraciji sedimentnih onesnaževal, kot predlagajo Field et al. (2002). Potencialna toksičnost organskih ekstraktov sedimentov bo merjena z uporabo bioanalize Microtox® (Bihari et al., 2007). Vrednosti EC50 bodo ocenjene z uporabo programske opreme MicrotoxOmniTM. Kronična toksičnost ekstraktov sedimentov bo določena z merjenjem *in vivo* inhibicije rasti morskega planktona *Dunaliella bioculata* (syn. *D. salina*) zaradi izpostavljenosti modelnim snovem (onesnaževalom) in ekstraktom v trajanju treh dni. Začetna gostota alg bo določena z optično gostoto alg (A 680 nm) z uporabo večfunkcionalnega bralnika mikroplati in spektrofotofluorimetra (Tecan Infinite M200 Pro s programom Magellan). Mikroalge bodo nato inkubirane v prisotnosti vzorca 72 ur pri sobni temperaturi. Optična gostota alg bo določena dnevno. Rezultati inhibicije rasti morskega planktona – *D. bioculata* bodo izračunani kot odstotek preživetja. Za test fitotoksicnosti bo 4 g suhega sedimenta eluiranih 24 ur z ultrapure vodo (1:10 sediment/voda) v skladu s Pelikan et al. (2022). Test kalitve bo izveden na semenu lana (*Linum usitatissimum*) pri 25°C za testno obdobje 72h (Mamindy-Pajany, 2011). Za vsak replikat (3) vzorca sedimenta bodo tri liste filtra postavljeni v 3 Petrijeve sklede (100 × 15 mm). Tri skupine po 30 semen bodo postavljene v vsako Petrijevo skledo in 5 ml eluata (ekvivalentno 100 g/L suhega sedimenta) ali ultrapure vode dodanih na filtre za vzorce sedimentov in kontrole, v skladu s tem. Petrijeve sklede bodo postavljene v vlažni termostat (HERA Cell 150, Heraeus) v temi. Po 24 urah bo določen odstotek kalitve semen (SIG).

Toksičnost in korelacija med rezultati preučevanih mest bo izvedena z uporabo STATISTICA 8.0 (StatSoft Inc, ZDA).

Rezultati in diskusija:

Kontrolni vzorec RV001 je dominiran s peskom (62.48%) in blatom (33.84%). Vzorci sedimentov CUVI-1, CUVI-2 in CUVI-3 so najfinejši sedimenti iz analiziranega sklopa, kjer je blato (vsebnost mulja in gline) dominantna frakcija (S7 64.33%, S8 54.20% in S9 68.88%) in višja vsebnost TOC, v skladu s tem (Tabela 4).

Vzorce porazdelitve PAH (3- do 6-obročni) kažejo, da prevladujejo PAH 4- in 5-obročki (70% na vseh mestih vzorčenja), kar je skladno s pirigenim izvorom (Tabela 5). Rezultati kažejo relativno nizko koncentracijo PAH v morskem sedimentu, zbranem na kontrolnem mestu RV001, kot tudi na mestih v bližini UPOV Cuvi.

Enotne koncentracije PCB (<0.10 mg/kg d.w.) izmerjene v površinskih sedimentih v tej študiji (Tabela 6) so bile nižje ali v razponu tistih, ki so bile prej odkrite v nepokvarjenem obalnem območju Rovinja 0.01 – 0.278 mg/kg d.w. (Pelikan et al., 2022). Več študij je pokazalo, da se PCB raje akumulirajo v finodeljenih frakcijah sedimentov, zato bi lahko pričakovali višjo koncentracijo ΣPCB v sedimentih Cuvi. Vendar pa lokalni učinki dejavnosti čiščenja odpadnih voda UPOV Cuvi niso privedli do višjih koncentracij PCB v vzorcih sedimentov (S7 – S9).

Koncentracije slednih kovin, najdenih v sedimenih, ustrezajo naravni vsebnosti v Jadranu (Martinčić et al., 1989; Ujević et al., 1998; Ferrara & Maserti, 1992; Bogner et al., 2005; Mikac et al., 2006) in so v razponu, značilnem za nedotaknjena do nepokvarjena območja Sredozemlja (UNEP, FAO, WHO, 1989; UNEP 1994; UNEP, 1996).

Verjetnost toksičnosti sedimentov je nizka za vsa mesta UPOV Cuvi (Pavg 0.19-0.22; Pmax 0.21-0.23), vendar višja kot za kontrolno mesto RV001 (Pavg 0.18; Pmax 0.15) (Tabela 7). Akutna toksičnost ekstraktov sedimentov je bila v razponu nepokvarjenih mest. Kronični toksični test z uporabo morskega fitoplanktona *D. salina* ne razlikuje sedimentov kontrolnega območja RV001 od sedimentov mest UPOV Cuvi (S7 – S9). Poleg tega pridobljene vrednosti EC50 kažejo na dobro okoljsko stanje morskih sedimentov v obalnem območju Rovinja. Jasno je rastoča fitotoksičnost (SIG) sedimentov v bližini UPOV Cuvi.

Skupne koncentracije in posamezne koncentracije PAH, skupnih PCB in TM so bile primerjane z nacionalnimi SQS, ERL in ERM mejami, kot tudi pravnimi ravnmi onesnaževal Francije SQS N1 in N2 (Tabela 10 in 11). Rezultati so pokazali, da so bile skupne koncentracije onesnaževal na vseh mestih znatno nižje od vrednosti ERL in ERM, kar postavlja sedimente v razpon, kjer ne pričakujemo škodljivih toksioloških učinkov.

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