ARPAT
Agenzia regionale per la protezione ambientale della Toscana

Decreto del Direttore generale nr. 60 del 31/03/2025

Proponente: *Marta Bachechi*

Affari Generali

Pubblicità/Pubblicazione: Atto soggetto a pubblicazione *integrale* (sito internet)

Visto per la pubblicazione - Il Direttore generale: Dott. Pietro Rubellini

Responsabile del procedimento: *Dr. Fabio Cioni*

Estensore: *Marta Bachechi*

Oggetto: Presa d'atto dell'accordo tra ARPAT e UNIFI denominato "WATERPATH"

ALLEGATI N.: 2

Denominazione	*Pubblicazione*	*Tipo Supporto*
Allegato "A" - accordo WATERPATH	sì	digitale
Allegato "B" - descrizione del progetto	sì	digitale

Natura dell'atto: *non immediatamente eseguibile*

Trattamento dati personali: *Sì* **Numerosità degli interessati:** *1 - 1.000*

Il Direttore generale

Vista la L.R. 22 giugno 2009, n. 30 e s.m.i., avente per oggetto "Nuova disciplina dell'Agenzia regionale per la protezione ambientale della Toscana (ARPAT)" ;

Richiamato il decreto del Presidente della Giunta Regionale n. 74 del 23.03.2021, con il quale il sottoscritto è nominato Direttore generale dell'Agenzia Regionale per la Protezione Ambientale della Toscana;

Considerata la decorrenza dell'incarico di cui sopra dal 1° maggio 2021;

Dato atto che con decreto del Direttore generale n. 50 del 05.03.2024 è stato adottato il Regolamento di organizzazione di ARPAT, ai sensi dell'art. 20 co. 3 della LRT n. 30/2009, (approvato dalla Giunta Regionale Toscana con delibera n. 968 del 05/08/2024), successivamente adeguato alla DGRT 968/24 con decreto del Direttore generale n. 167 del 05.09.2024;

Visto l'"Atto di disciplina dell'organizzazione interna" approvato con decreto del Direttore generale n. 270/2011, modificato ed integrato con decreti n. 87 del 18.05.2012 e n. 2 del 04.01.2013, nonché l'"Atto di disciplina dell'organizzazione interna" approvato con decreto del Direttore generale n. 225 del 27.11.2024 in corso di attuazione;

Considerato che UNIFI è beneficiaria di un finanziamento nell'ambito della Water4All Joint Transnational Call 2023 – "Aquatic ecosystem services", per l'esecuzione del progetto denominato "Understanding and improving water-driven ecosystem services for the production of drinking water and treated wastewater for agricultural reuse, acronimo "WATERPATH" (il "Progetto"), della durata di 36 mesi e di cui l'Università è coordinatore;

Considerato che il Progetto prevede il coinvolgimento di 5 beneficiari transnazionali (il Consorzio") ed è finanziato dall'ente di finanziamento nazionale per ogni paese partecipante. In particolare, UNIFI sarà finanziata dal MUR – Ministero dell'Università e della Ricerca, con apposito Atto d'Obbligo che sarà stipulato al termine della negoziazione MUR/INVITALIA;

Ricordato che per contribuire al raggiungimento degli obiettivi strategici del Progetto, il Consorzio si avvarrà della partecipazione di ARPAT, come partner autofinanziato (Self-funded Partner A / SFP-A) che, tenuto conto delle proprie attività istituzionali obbligatorie e della propria struttura e quantificazione di bilancio, fornirà al Consorzio capacità di alto livello nel campionamento delle acque interne e marine.

Dato atto che ARPAT collaborerà con UNIFI e con il Consorzio, come indicato nell'Allegato Tecnico del Progetto, nella realizzazione delle attività sinteticamente di seguito descritte:

A) Obiettivo Realizzativo (OR) 2 - Monitoraggio spaziale e temporale della contaminazione chimica e biologica:
   Task 2.1 – Campionamento dell'acqua (M4-24
   Task 2.2 – NTA di CEC/CEC-TP chimici mediante LC-HRMS (M4-26)
   Task 2.3 – Screening delle microplastiche (M4-26)
   Task 2.4 – Analisi mirata di CEC/CEC-TP mediante LC-MS/MS (M7-29)
   Task 2.5 – Analisi target delle microplastiche mediante Py-GC-MS (M7-29)

B) OR5 – Comunicazione, diffusione e sfruttamento dei risultati
   Task 5.2 – Sviluppo delle capacità (M12-36), di cui ARPAT è task leader.
   Task 5.6 – Politica e legislazione (M12-36), di cui ARPAT è task leader.

C) ARPAT è responsabile del Deliverable D.5.6.1 – Rapporto sui contributi alle politiche e alla legislazione (policy brief) (M24,M36).

Visto il decreto del Direttore generale n. 192 del 30.12.2015 avente ad oggetto "Modifica del decreto del Direttore generale n. 138 del 26.09.2013 e adozione del "Disciplinare interno in materia di gestione dei rapporti tra le strutture di ARPAT ed il Collegio dei revisori";

Visto il parere positivo di regolarità contabile in esito alla corretta quantificazione ed imputazione degli effetti contabili del provvedimento sul bilancio e sul patrimonio dell'Agenzia espresso dal Responsabile del Settore Bilancio e contabilità riportato in calce;

Visto il parere positivo di conformità formale alle norme vigenti, espresso dal Responsabile del Settore Affari generali, riportato in calce;

Visti i pareri espressi in calce dal Direttore amministrativo e dal Direttore tecnico;

decreta

1. di prendere atto dell'accordo sottoscritto tra ARPATe UNIFI, allegato al presente provvedimento quale parte integrante e sostanziale (allegato "A") e della descrizione del progetto (allegato "B");
2. di individuare responsabile del procedimento il Dr. Fabio Cioni ai sensi dell'art. 4 della L. n. 241 del 07.08.1990 e s.m.i

Il Direttore generale
Dott. Pietro Rubellini*

"Documento informatico sottoscritto con firma digitale ai sensi del D.Lgs 82/2005. L'originale informatico è stato predisposto e conservato presso ARPAT in conformità alle regole tecniche di cui all'art. 71 del D.Lgs 82/2005. Nella copia analogica la sottoscrizione con firma autografa è sostituita dall'indicazione a stampa del nominativo del soggetto responsabile secondo le disposizioni di cui all'art. 3 del D.Lgs 39/1993."

Il Decreto è stato firmato elettronicamente da:

- Marta Bachechi , responsabile del settore Affari generali in data 26/03/2025
- Andrea Rossi , responsabile del settore Bilancio e Contabilità in data 26/03/2025
- Marta Bachechi , il proponente in data 27/03/2025
- Paola Querci , Direttore amministrativo in data 28/03/2025
- Marcello Mossa Verre , Direttore tecnico in data 28/03/2025
- Pietro Rubellini , Direttore generale in data 31/03/2025

ACCORDO CON PARTNER AUTOFINANZIATO

Tra

Università degli Studi di Firenze – Dipartimento di Chimica "Ugo Schiff", con sede legale in Firenze, P.zza San Marco n. 4, codice fiscale e partita IVA: 01279680480, rappresentata dal Direttore del Dipartimento Prof. Stefano Menichetti, di seguito indicato come "Beneficiario" o "UNIFI"

e

Agenzia Regionale per la Protezione Ambientale della Toscana, con sede legale in Via Ponte alle Mosse n. 211, 50144, Firenze, codice fiscale e partita IVA: 04686190481, rappresentata dal Direttore Generale Pietro Rubellini, di seguito indicata come "ARPAT"

congiuntamente anche "le Parti"

PREMESSO CHE

1. UNIFI è beneficiaria di un finanziamento nell'ambito della Water4All Joint Transnational Call 2023 – "Aquatic ecosystem services", per l'esecuzione del progetto denominato "Understanding and improving water-driven ecosystem services for the production of drinking water and treated wastewater for agricultural reuse, acronimo "WATERPATH" (il "Progetto"), della durata di 36 mesi e di cui l'Università è coordinatore;
2. il Progetto prevede il coinvolgimento di 5 beneficiari transnazionali (il Consorzio") ed è finanziato dall'ente di finanziamento nazionale per ogni paese partecipante. In particolare, UNIFI sarà finanziata dal MUR – Ministero dell'Università e della Ricerca, con apposito Atto d'Obbligo che sarà stipulato al termine della negoziazione MUR/INVITALIA;
3. per contribuire al raggiungimento degli obiettivi strategici del Progetto, il Consorzio si avvarrà della partecipazione di ARPAT, che ha fornito lettera di sostegno, come partner autofinanziato (Self-funded Partner A / SFP-A), fornendo al Consorzio capacità di alto livello nel campionamento delle acque interne e marine.

Tutto ciò premesso,

SI CONVIENE E STIPULA QUANTO SEGUE

con le premesse e gli allegati facenti parte integrante dell'accordo:

Art. 1 – Interpretazione e rinvio

1. Il presente accordo è collegato all'Atto d'Obbligo ed è stipulato per consentire l'adempimento delle obbligazioni di UNIFI nei confronti del MUR e del Consorzio in relazione al Progetto.
2. ARPAT dichiara di essere a conoscenza dell'Allegato Tecnico del Progetto e di averne ricevuto una copia.

Art. 2 – Obblighi di ARPAT

1. ARPAT collaborerà con UNIFI e con il Consorzio, come indicato nell'Allegato Tecnico del Progetto, nella realizzazione delle attività di seguito descritte:

A) Obiettivo Realizzativo (OR) 2 - Monitoraggio spaziale e temporale della contaminazione chimica e biologica:

Task 2.1 – Campionamento dell'acqua (M4-24)

Le attività di campionamento riguarderanno la raccolta di campioni di acqua per le analisi descritte nei Task 2.2-2.6. Le CEC saranno studiate nei seguenti servizi ecosistemici rilevanti: (i) tre bacini fluviali (Po e Arno in Italia e Mures in Romania), includendo nel monitoraggio i punti di presa d'acqua per la produzione di acqua potabile, i punti di scarico di effluenti degli impianti di depurazione, nonché i punti a monte e a valle di tali scarichi, nonché una serie di altri punti rappresentanti la raccolta delle acque a scopo irriguo; (ii) il bacino idrico sotterraneo del pistoiese (Italia) utilizzato per la produzione di acqua potabile e per l'irrigazione; (iii) tre aree marine costiere, con diverse posizioni, caratteristiche e fonti di inquinamento di origine antropica, vale a dire Las Canteras, Arinaga e Playa del Inglés, situate rispettivamente sulle coste nord-est, sud-est e sud dell'isola di Gran Canaria , che rappresentano le aree in cui i tre impianti di depurazione dell'isola scaricano i loro TWW, ma anche zone di produzione di acqua potabile mediante desalinizzazione. Il monitoraggio del suddetto punto di campionamento per NTA sarà eseguito su una scala temporale quadrimestrale per i primi due anni di progetto (ovvero sei campionamenti), ottenendo così un quadro sia spaziale che temporale della presenza di CEC/CEC-TP.

Task 2.2 – NTA di CEC/CEC-TP chimici mediante LC-HRMS (M4-26)

I campioni raccolti come spiegato nella Sezione 4 verranno elaborati per NTA mediante (i) esperimenti di scansione completa LC-HRMS seguiti da (ii) esperimenti di massa tandem ad alta risoluzione (LC-MS/HRMS) per la delucidazione strutturale e (iii) livello I o identificazione di livello II (vedere Sezione 4 per ulteriori dettagli). Verranno stabiliti protocolli NTA tra i partner al fine di ottenere procedure analitiche comuni e un'elevata qualità dei dati.

Task 2.3 – Screening delle microplastiche (M4-26)

I campioni raccolti nell'Attività 2.1 saranno pretrattati mediante la combinazione ottimale di protocolli di separazione di densità e ossidazione al fine di estrarre le microplastiche e separarle dalle interferenze. Le frazioni estratte verranno dapprima caratterizzate mediante osservazione visiva mediante stereomicroscopio, classificando i frammenti in base alla morfologia, alle proprietà ottiche e al comportamento meccanico; ulteriore conferma verrà effettuata mediante Py-GC-MS.

Task 2.4 – Analisi mirata di CEC/CEC-TP mediante LC-MS/MS (M7-29)

Una volta ottenuti gli elenchi CEC/CEC-TP, verranno eseguiti flussi di lavoro di analisi MS/MS mirati basati su strategie di acquisizione sia LC-HR-MRM che LC-MRM per valutare quantitativamente la presenza di CEC nei campioni di acqua selezionati. Per le CEC con standard analitici di riferimento disponibili, verranno utilizzati protocolli di quantificazione basati su curve di calibrazione esterne e/o abbinate a matrice. Viceversa, in caso di indisponibilità di standard analitico, verrà eseguita una procedura semiquantitativa surrogata [16]. Anche all'interno di questa attività, saranno definiti protocolli per la selezione di metodi quantitativi, per aumentare la quantificazione della CEC in base alle esigenze di ciascuna matrice indagata (ad esempio, sensibilità ed effetto matrice)

Task 2.5 – Analisi target delle microplastiche mediante Py-GC-MS (M7-29)

Per ciascun polimero identificato nell'Attività 2.3, verranno selezionati i picchi di pirolisi e gli ioni caratteristici più specifici. Gli ioni di quantificazione ottenuti verranno infine utilizzati per costruire una curva di calibrazione esterna utilizzando standard di riferimento microplastici per ciascun polimero per valutare quantitativamente la presenza di microplastica nei campioni di acqua selezionati.

B) OR5 – Comunicazione, diffusione e sfruttamento dei risultati:

Task 5.2 – Sviluppo delle capacità (M12-36), di cui ARPAT è task leader.

Una strategia di rafforzamento delle capacità sarà progettata e inclusa nel PMH.

Task 5.6 – Politica e legislazione (M12-36), di cui ARPAT è task leader.

Questo compito mira a informare i decisori politici sui risultati di WATERPATH, supportando le azioni di politica ambientale e aggiornando le liste di priorità e di monitoraggio della comunità europea. Questo compito mira anche a informare gli attori del processo decisionale politico sui risultati relativi al riciclo dei fanghi biologici per la produzione di biochar, contribuendo al processo di end-of-waste di questi rifiuti, secondo la recente Direttiva Europea sui Rifiuti Direttiva quadro 2018/851, che definisce lo strumento giuridico dell’end-of-waste in un’ottica di economia circolare. Un documento programmatico sarà redatto con l’assistenza degli attori politici che hanno firmato lettere di sostegno.

C) ARPAT è responsabile del Deliverable D.5.6.1 – Rapporto sui contributi alle politiche e alla legislazione (policy brief) (M24,M36).

2. ARPAT si obbliga in modo specifico a:

a) svolgere l'attività di cui al precedente comma, tenuto conto del particolare contenuto dell'attività e tenendo in considerazione che detta attività è necessaria per adempiere le obbligazioni che nascono dall'Atto d'Obbligo;

b) redigere i deliverable e le attività ad essa attribuite entro i termini indicati nell'Allegato tecnico del Progetto;

c) rispettare le direttive di massima di UNIFI emanate per l'attuazione del Progetto, nelle fasi descritte nell'Allegato Tecnico al Progetto;

L'Agenzia, tenuto conto delle proprie attività istituzionali obbligatorie e della propria struttura e quantificazione di bilancio, svolgerà la propria attività nell'ambito del progetto compatibilmente con le stesse. L'agenzia si impegna comunque a comunicare a UNIFI, nel più breve tempo possibile, tutti gli eventi che riguardino il presente accordo e in modo particolare quelli che possano compromettere l'esatta esecuzione dell'attività.

Art. 4 – Responsabilità di ARPAT

ARPAT è responsabile per tutti i danni derivanti ad UNIFI ed al Consorzio dallo svolgimento, o dal mancato svolgimento, delle sue attività di Progetto.

Art. 5 – Contributi

ARPAT partecipa al Progetto quale partner autofinanziato, senza il diritto di addebitare costi o richiedere contributi.

Art. 6 – Responsabili scientifici

1. ARPAT individua il Dott. Cioni Fabio quale responsabile scientifico, con incarico della direzione e del coordinamento di tutte le attività previste.

2. UNIFI individua il Prof. Massimo del Bubba quale supervisore della ricerca affidata.

Art. 7 – Durata

1. Il presente accordo entrerà in vigore alla data della firma dell'ultima Parte e resterà in vigore fino alla fine del Progetto.

2. In caso di concessione di eventuali proroghe alla durata del Progetto, è conseguentemente prorogata anche la durata del presente accordo e UNIFI si impegna a darne comunicazione per iscritto ad ARPAT.

3. La risoluzione del presente accordo non pregiudica i diritti o gli obblighi di ARPAT sorti prima della data di risoluzione. Ciò include l'obbligo di fornire tutte le relazioni, i report e tutta la documentazione necessaria per le relazioni inerenti allo svolgimento delle task cui ARPAT partecipa.

4. UNIFI potrà risolvere l'accordo, a seguito dell'inadempimento o della violazione delle obbligazioni gravanti su ARPAT, dandone un preavviso di almeno quindici giorni, a mezzo pec, nella quale si dichiara la volontà di esercitare il diritto di cui al presente articolo.

Art. 8 – Proprietà industriale e intellettuale dei Risultati e diritti di accesso

1. I Risultati del Progetto sono di proprietà del Consorzio.

2. Ciascuna Parte è titolare dei diritti di proprietà intellettuale e industriale relativi al proprio Background.

3. I diritti di accesso ai Risultati e al Background necessari per l'implementazione del lavoro di una Parte, ai sensi del Progetto, saranno concessi all'altra Parte e, per quanto riguarda ARPAT, agli altri partner del Consorzio, a titolo gratuito, salvo diverso accordo scritto.

4. Inoltre, fino a dodici mesi dopo la fine del Progetto, ARPAT si impegna a rendere accessibili ai partner del Consorzio il proprio Background ed i propri Risultati, se necessari per lo sfruttamento dei Risultati di questi ultimi, a condizioni eque e ragionevoli. I diritti di accesso ai Risultati per attività di ricerca interna non commerciale e per scopi didattici non commerciali saranno concessi a titolo gratuito.

Art. 9 - Disseminazione e open science

1. ARPAT prende atto che il Consorzio adotterà la pratica "Open Access" di fornire accesso online a informazioni scientifiche che siano gratuite e riutilizzabili per l'utente. Ciò includerà pubblicazioni sottoposte a revisione paritaria, ma anche dati e set di dati WATERPATH, seguendo il principio FAIR (Findable, Accessible, Interoperable and Re-usable). Verrà preparato un Data Management Plan (DMP) secondo l'approccio FAIR come parte del "Project Management Handbook" (vedere Task 1.2), che descrive in dettaglio quali dati genererà il Progetto e come saranno sfruttati e archiviati. Verrà creata una semplice piattaforma di collaborazione per archiviare e condividere dati, informazioni, scambiare messaggi e lavorare sui risultati. Una prima versione del DMP verrà consegnata entro i primi 3 mesi del progetto. Poiché si prevede che il DMP maturi durante il progetto, saranno incluse altre versioni in fasi successive.

2. Fatto salvo quanto sopra, qualora ARPAT intenda divulgare i Risultati del Progetto, potrà farlo solo previa autorizzazione scritta di UNIFI, a cui sarà sottoposto il testo da pubblicare almeno 60 giorni prima della divulgazione.

Art. 10 - Riservatezza

1. Tutte le informazioni in qualsiasi forma o modalità, che sono divulgate da una Parte (la "Parte Divulgante") all'altra Parte (il "Destinatario") in relazione al presente accordo e alla sua esecuzione e che è stato esplicitamente contrassegnato come "riservato" al momento della divulgazione o, quando divulgato oralmente, è stato identificato come riservato al momento della divulgazione e ciò è stato confermato per iscritto entro 15 giorni di calendario dalla divulgazione orale, sono "Informazioni riservate".

2. Il Destinatario si impegna per un periodo di 5 anni dopo il pagamento finale da parte dell'Ente Finanziatore a:

- non utilizzare le Informazioni riservate se non per lo scopo per il quale sono state divulgate;

- non divulgare Informazioni Riservate senza il previo consenso scritto della Parte Divulgante;

- garantire che la distribuzione interna delle Informazioni riservate da parte di un Destinatario avvenga in base a rigorose esigenze di conoscenza; e

- restituire alla Parte Divulgante, o distruggere, su richiesta, tutte le Informazioni Riservate che sono state divulgate al Destinatario, comprese tutte le loro copie, e cancellare tutte le informazioni memorizzate in un formato leggibile dalla macchina per quanto praticamente possibile. Il Destinatario può conservare una copia nella misura in cui è necessario per conservare, archiviare o conservare tali Informazioni riservate a causa della conformità alle leggi e ai regolamenti applicabili o per la prova degli obblighi in corso a condizione che il Destinatario rispetti gli obblighi di riservatezza qui contenuti in relazione a tale copia per tutto il tempo in cui la copia viene conservata.

3. Il Destinatario sarà responsabile dell'adempimento degli obblighi di cui sopra da parte dei propri dipendenti o di terzi coinvolti nell'espletamento dei compiti e si assicurerà che rimangano obbligati, per quanto legalmente possibile, durante e dopo la cessazione del presente accordo e/o dopo la cessazione del rapporto contrattuale con il dipendente o con terzi.

4. Quanto sopra non si applica alla divulgazione o all'uso di Informazioni riservate, se e nella misura in cui il Destinatario può dimostrare che:

- le Informazioni riservate sono diventate o sono rese disponibili pubblicamente con altri mezzi che una violazione degli obblighi di riservatezza del Destinatario;

- la Parte Divulgante informa successivamente il Destinatario che le Informazioni Riservate sono non più riservate;

- le Informazioni Riservate sono già in possesso del Destinatario prima della data della firma del presente accordo o vengono comunicate al Destinatario senza alcun obbligo di riservatezza da parte di un terzo; o

- le Informazioni riservate, in qualsiasi momento, sono state sviluppate completamente dal Destinatario indipendentemente da tale divulgazione da parte della Parte Divulgante;

- il Destinatario è tenuto a divulgare le Informazioni Riservate al fine di ottemperare a leggi o regolamenti applicabili o con un ordine del tribunale o amministrativo.

5. Il Destinatario applicherà lo stesso grado di attenzione per quanto riguarda le Informazioni Riservate divulgate come per le proprie Informazioni Riservate.

6. Ciascuna Parte avviserà prontamente l'altra Parte per iscritto di qualsiasi divulgazione non autorizzata, appropriazione indebita o uso improprio delle Informazioni Riservate dopo che è venuta a conoscenza di tale divulgazione non autorizzata, appropriazione indebita o uso improprio.

Art. 11 – Copertura assicurativa

1. Ciascuna Parte provvederà alla copertura assicurativa per il rischio infortuni del proprio personale.

Art. 12 – Controversie e legge applicabile

1. Il presente accordo deve essere interpretato in conformità e disciplinato dalla legge italiana.

2. Qualsiasi controversia che dovesse nascere dall'esecuzione del presente accordo, qualora le Parti non riescano a definirla amichevolmente, sarà devoluta all'Autorità giudiziaria competente.

3. L'Autorità giudiziaria competente è il Giudice amministrativo quale giurisdizione esclusiva ai sensi dell'art. 133 del decreto legislativo 2 luglio 2010, n. 104.

Art. 13 – Registrazione e imposta di bollo

1. Il presente atto è soggetto a registrazione in caso d'uso ai sensi dell'art. 5 punto 1 del D.P.R. 131/86 e dell'art. 4 della tariffa – Parte II - annessa al medesimo decreto, a cura e spese della parte richiedente.

2. Le spese relative all'imposta di bollo, ai sensi dell'art. 2 all. A Tariffa, parte 1° DPR 642/1972, sono poste a carico del Beneficiario.

Letto, confermato e sottoscritto digitalmente.

Agenzia Regionale per la Protezione Ambientale
della Toscana
Il Direttore Generale
Pietro Rubellini

Università degli Studi di Firenze
Dipartimento di Chimica
Il Direttore
Prof. Stefano Menichetti

Water4All
Water security for the planet

Water4All 2023 Joint Transnational Call on "Aquatic Ecosystem Services"

Full proposal evaluation Water4All2023-790 (WATERPATH)

Dear Massimo Del Bubba,

The Water4All Call Secretariat, on behalf of the Call Steering Committee, wishes to thank you for the submission of the proposal Water4All2023-790 (WATERPATH) in response to the Water4All 2023 Joint Transnational Call on "Aquatic Ecosystem Services".

A total of 59 proposals were submitted to this call.

We are pleased to inform you that this proposal has been **recommended for funding**. This decision was based on the scientific evaluation of the proposals by the International Evaluation Panel and the budget availability.

Please consider below:

i. the comments related to the eligibility checks conducted by the Call Secretariat and the funding organisations;
ii. the common consensus report of your proposal.

i. Results of the eligibility check

Decision: **eligible**

ii. Full proposal evaluation summary

Criterion 1. Excellence

The proposed work is highly ambitious. The novelty is outlined in the proposal and includes testing of novel methods for non-target analysis of Contaminants of Emerging Concern (CEC) and the combination of quantitative CEC and biological data, collated in a comprehensive database in three contrasting but complementary geographical areas in Europe. The proposal will further give quantitative understanding of the impact of multiple CECs on multiple ecosystem services, including Antibiotic Resistance Genes (ARGs) in potable and irrigation water. The research has a good interdisciplinary character with several disciplines involved, including chemistry, microbiology and chemical engineering.
The proposal is highly ambitious and takes scientific risks in terms of developing new approaches to non-target analysis of CECs and their quantification. However, these risks are addressed and mitigation measures are outlined. The most ambitious and novel aspects of the research are the

three model geographical areas covering different characteristics in terms of ecosystems services. The use of nanofiltration membranes and constructed wetland that integrates biochar is also not truly innovative in the field of wastewater treatment.
The methodology and research design are clear, feasible and suitable to answer the identified knowledge gaps and/or achieve the proposed objectives. Research design and methods are described in detail. A minor shortcoming appears to be lack of the number of sampling locations anticipated in each study area and hence an estimation of the final size of the database that will be generated.
WATERPATH will adopt the "Open Access" practice of providing online access to scientific information that is free of charge and reusable to the user. This will include peer-reviewed publications, but also WATERPATH data and datasets, following the FAIR (Findable, Accessible, Interoperable and Re-usable) principle.
Although WATERPATH is an advanced research project, the topics covered can be the subject of appropriate communication activities towards citizens, civil society, and end users of the ecosystem services studied.
Gender dimension in research and innovation is not addressed, which is a shortcoming.

Criterion 2. Impact

The WATERPATH consortium pursues the objective to provide large-scale quali-quantitative information on the pressure of CECs on key ecosystem services in geographically distinct but complementary areas, thus providing relevant information for improving ecosystem governance for the provision and protection of related ecosystem services to policymakers within the consortium geographical areas. In each geographical area, WATERPATH will provide comprehensive data on different ecosystems and ecosystem services pursuing a concept of integration of ecosystem services approaches. The accurate knowledge of CECs/CEC-TPs and ARB/ARGs in the ecosystems studied undoubtedly represents the first and essential step to understand which technical and political strategies must be adopted to alleviate anthropogenic pressure on these ecosystems, thus contributing to the restoration of natural ecosystems for the conservation of ecosystem services.
The plan for impact is detailed, ambitious and follows logically the expected results of the project. Strategic impact is addressed as the proposal responds directly to an EU Directive that will require member states to carry out risk assessments that cover the whole drinking water supply chain. The results of this work will inform the EU Priority Lists, the Monitoring Watch Lists and regional Water Protection Plans. Measures to maximise expected outcomes and impacts, including communication activities are set out and clearly targetted at different audiences - from scientific, to policy, water management and the general public.
The proposal identifies a range of actors to make successful use of the results. These are listed in detail and many have already provided letters of support. Relevant stakeholders will also be represented on the Project Management Board. The Management structure of the consortium is clear and specified in detail. A Dissemination and Exploitation and Communication Board and three General Assembly meetings are envisaged to communicate results to stakeholders and society.
There is a clear communication plan using dissemination, exploitation and communication tools:

website, Social Media, Newsletters and public engagement (oriented to high-school students). WATERPATH considers three model geographical areas (i.e., continental, peninsular and insular) with complementary features in terms of suffered anthropic pressure (i.e., industrial, agricultural and tourist impacts) and ecosystem services under investigation (for example, drinking water from groundwater, river or sea water), thus providing results that go beyond the specific locations investigated and can be transferred to other environmental scenarios and related ecosystem services. WATERPATH intends to pursue transnational added value through the collaboration with ongoing international initiatives regarding CEC/CEC-TP and/or ARB/ARG spreading in ecosystems, such as the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR), which involves 19 European countries.
The economic impacts are not well developed although they are proposal relevant. The proposal does not give a clear outline of how the research may lead to new know-how and treatment technology relevant for the water market.

Criterion 3. Quality and efficiency of the implementation

The consortium brings together the necessary expertise with a strong track record. This proposal builds on prior collaborations within the consortium and a solid track record in the research disciplines. The participants in the proposal are well-suited to their tasks, their roles are well-defined and complement each other well, although a social science expertise is missing from the consortium. The consortium includes both academic and non-academic partners, including an environmental regulator and a water management company. The tasks are well balanced among partners.
The proposed organization and management of the scientific project is outlined in detail. It is effective and efficient. The management structures and procedures, including innovation management are appropriate. The proposal does acknowledge certain risks related to the research (Table 2), but there is room for improvement in explicitly addressing these risks and outlining contingency measures. The contingency plans to tackle the risks are too generic and not convincing.
A minor shortcoming appears to be lack of detailed Justification of Resources by the leading Project Partner. Overall, the resources appear modest, compared to the ambition of the project - this includes the resources for outreach and communication.
Regarding personnel costs of Partner 1 and 2, the origin and the degree of certainty of their own funding (85k€ and 51.5 k€, respectively) is not provided. Regarding partner 2, the cost for consumables is very low (9.2 k€) given their high involvement in experimental work.

Please note that the definitive funding decision will depend on the following:

- Outcomes of the Redress procedure;
- The final approval of the shortlisted proposals by the boards of the participating Funding Organisations.

Please note that this letter does not guarantee funding or funding without any budget cuts.
The Call Secretariat will confirm the final funding decision in a separate communication once the

above-mentioned steps have been finalized. Please note that in parallel, some Funding Organisations may require further (financial) information on their respective proposals. The Funding Agencies will contact their respective project partners as soon as possible regarding the national granting procedure requirements.

The final list of proposals granted funding will be published on Water4All European Partnership website, https://www.water4all-partnership.eu/ , and possibly on national websites.

We remind you that the consortia of funded projects are expected to ensure a correct project management in line with Art. §7 of the call text, including the preparation and submission of a Consortium Agreement before commencement of the project and the attendance of the project Kick-off, Mid-term and Final evaluation meetings.

Please note that the proposal coordinator is responsible for informing all consortium participants about this decision.

Yours sincerely,
The Water4All Call Secretariat
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Understanding and improving water-driven ecosystem services for the production of drinking water and treated wastewater for agricultural reuse (WATERPATH)

1. State of the art, own work, previous activities of the consortium in the field.

1A. State of the art - *Introduction to the problem*. The ever-increasing production and use of chemicals worldwide represent a real threat, generating a negative anthropic pressure on aquatic ecosystems with obvious consequences on the quality of the ecosystem services provided, such as raw water to be treated for drinking purposes or to be used for the irrigation of edible crops. Indeed, man-made activities release/produce chemical contaminants of emerging concern (CECs) and/or biological contamination in surface and/or groundwaters through both point (e.g., wastewater treatment plants, WWTPs) and nonpoint (e.g., agriculture and tourism) sources, thus potentially affecting also the production of both edible crops and drinking water, and thus humans as the final target [**1,2**]. CECs are substances that have been detected in the environment with completely or partially unknown (eco)toxicological effects, such as surfactants, pharmaceuticals, biocides, UV-filters, together with their transformation products (CEC-TPs), and microplastics [**3**]. Antibiotic resistance bacteria and/or genes (ARB and ARGs) are also considered as CECs [**4,5**]. *Identifying the chemical CEC/CEC-TP status*. Given the (i) high variability of water ecosystems and related services and the (ii) challenging nature of monitoring the "cocktail" of thousands of CECs/CEC-TPs in water bodies and depuration/potabilization facilities, non-targeted analysis (NTA) platforms based on liquid chromatography (LC) coupled with high-resolution mass spectrometry (HRMS) represent the techniques of choice in analytical chemistry to respond to the urgent need of comprehensive qualitative data on the chemical health status of aquatic ecosystems [**6,7**]. The key analytical traits of chemical NTA workflows, such as accurate mass-to-charge ratio (m/z) of both precursor and fragment ions combined with a specific retention time (t_R), are suitable to generate highly informative chemical datasets for CEC/CEC-TP annotation [**8**]. These workflows generally involve the solid phase extraction (SPE) of a few hundreds of mL of water samples, using multiple interaction and mixed-mode sorbents followed by cartridge elution with proper solvents and volume reduction to increase the sensitivity of the acquisition [**9-11**]. However, very little focus has been placed on upstream optimization of specific analytical parameters (e.g., chromatographic conditions and ion source parameters), thus allowing for further increase the overall sensitivity of the method and therefore the detection rate of CECs in NTA experiments. In fact, sensitivity may represent a weak point of NTA approaches. LC-HRMS-based NTA has been widely employed for obtaining qualitative information on CEC occurrence in various kinds of aquatic environments [**1**]. Nevertheless, these studies usually aim to detect the presence of CECs in the environment, while only in a few cases they had the more ambitious objective of studying the migration of CECs from a point source towards an ecosystem service [**11**]. Moreover, a quite recent review [**12**] raised some major concerns about future directions of the research in the field of CEC monitoring, focusing, among other things, on the facts that CEC-TP formation is often ignored, even if TPs may be more toxic than precursors. If LC-HRMS is the election technique for the analysis of CECs at molecular level, Py-GC-MS is one of the most promising approaches for the detection of microplastics, since mixtures of polymers could be simultaneously identified, isolating also the contribution of plastic additives. Indeed, despite some issues of cross-interferences [**13**] and matrix-dependent limited reproducibility [**14**], this instrumental approach has demonstrated its validity for the screening of MPs in the environmental compartments [**15**]. *Quantifying the chemical CEC/CEC-TP status*. It should also be noted that to achieve a better understanding of the pressure of CECs on aquatic ecosystems, i.e., the potential risk of worsening the quality of related ecosystem services, it is also necessary to combine NTA datasets with quantitative data. This is also of paramount importance when the study aims at interpreting the variation of the data on a spatial and temporal scale, especially if the large number of features that are usually annotated in NTA studies must be considered [**16**]. To this aim, targeted analyses via LC-HRMS, Py-GC-MS, and both high- and low-resolution tandem mass spectrometry (MS/MS) can be carried out, measuring reference standards under the same experimental conditions of sample analysis (level I of identification) to provide high-quality quantitative outputs. Additionally, a semi-quantitative analysis can be performed for the screened CECs lacking reference standards (and therefore identified at level II, i.e. thanks to t_R and MS/MS structural information) using surrogate standards chosen based on t_R data, mass spectra, and response factors; in fact, the most accurate results from surrogate applications have been observed when considering chemicals within a defined chemical class, i.e., with very similar mass fragmentation and close t_R values [**17**]. Even though it is possible to implement analytical protocols for the quantification of CECs/CEC-TPs using extensive targeted analyses as a follow-up of chemical NTA, these workflows require demanding efforts in terms of cost and time to be able to extend targeted quantification to the vast range of CECs/CEC-TPs previously identified in non-target monitoring. Thus, available quantitative studies regard in all cases a limited coverage of chemical space in terms of CEC use categories and/or number of analytes targeted in a certain category, while there are very few examples integrating NTA and targeted protocols for risk assessment of CECs in surface water and wastewater [**18,19**]. It should also be noted that these researches investigate ecosystems that are highly circumscribed and poorly representative of the risk, impact, and pressure of the related ecosystem services. *__Identifying and quantifying the biological ARB/ARGs status__*. Selective pressure of CECs/CEC-TPs on ARB/ARGs from water is of relevant health concern. In fact, ARB/ARGs selected by CECs/CEC-TPs and transferred by polluted water ecosystems could disseminate in the human and animal microbiome [**20**]. The link between CECs and the resistome has been thoroughly explored in other settings, such as the human gut [**21,22**]. On the contrary, a limited number of studies have suggested a connection between CECs/CEC-TPs and the microbial resistome in aquatic ecosystems [**23,24**]. Accordingly, future research should focus on identifying selective pressures from CECs/CEC-TPs in aquatic environments that influence the development and spread of ARGs and ARB, as well as understanding how organisms bypass taxonomic barriers to transfer ARGs. The current literature also reports heterogeneity about methodologies, study designs and analytical strategies used to correlate CECs with ARGs/ARB in unique aquatic environments [**25**]. This heterogeneity complicates the identification of consistent patterns, such as possible dose-response relationships in different water ecosystems. Therefore, it is crucial to conduct comparative research across various environments using a standardised experimental framework to discern patterns in CEC/CEC-TP interactions with ARGs or ARB. The complexity of these research questions necessitates advanced methodologies, such as targeted and untargeted metagenomics, which have become powerful tools for studying the metataxonomy and resistome across diverse environments [**26,27**]. Sequencing technologies have progressed to next-generation sequencing (NGS) and third-generation sequencing (TGS) like PacBio SMRT (Single-Molecule Sequencing in Real Time), enhancing the coverage precision and breadth of metagenomic analysis. PacBio sequencing, in particular, is advantageous for assembling complex metagenomes [**28**]. However, the development of further robust bioinformatics tools is needed to improve assemblies and detect low-abundance ARGs. In addition, biomarkers, such as specific ARGs (or other genes) have been recently proposed as indicators of chemical contamination and/or used to evaluate mitigation strategies in water systems, contributing to assess pollution levels and the effectiveness of bioremediation actions [**29,30**]. Nevertheless, the focus on the parallel monitoring of the microbiological and chemical contaminations is still poorly described, especially using comprehensive chemical and microbiological approaches [**31**]. Cultivable microorganisms also complement metagenomic studies by confirming the identifications from the metagenomes, providing a better understanding of ARG distribution in living microbial communities [**32**]. The cultivation approach is cost-effective and allows for a detailed comparison of resistance profiles, shedding light on the genetic basis of resistance in cultivable bacteria, including their potential for horizontal gene transfer [**33**]. Cultivation approach may also specifically address multidrug-resistant bacteria in waters, ARG dissemination via mobile genetic elements, and the role of integrons in antibiotic resistance spread in natural bacterial populations. Integrating metagenomic and cultivable methods may deepen the understanding of transposons and their role in ARG transfer. Cultivable bacteria facilitate targeted analysis of transposons in controlled lab conditions, allowing for direct manipulation and genetic characterization [**34**]. *__The mutual integration of chemical and biological status__*. A significant challenge in the current literature is the limited integration of chemical and biological data within ecosystems, particularly in the context of the evaluation of anthropogenic pressures. Wastewater often contains CECs/CEC-TPs (e.g., antibiotics) due to their extensive use, promoting the proliferation of ARB/ARGs through mutations and horizontal gene transfer, the latter considered to be the most important factor in the current pandemic of antimicrobial resistance [**35**]. This means that chemical-related ARGs can spread among different bacterial species and communities in wastewaters and environmental waters. The parallel monitoring of the prevalence and diversity of CECs/CEC-TPs and ARB/ARGs in wastewater in longitudinal studies give an indication of the potential risk for the spread of antibiotic resistance. Although the mutual integration of chemical and biological data through multivariate analysis (e.g., meta-analysis) has been already demonstrated, the few studies carried out relate only to semi-quantitative data (such as detection frequency) of CECs/CEC-TPs and cultivable microorganisms, while studies based on chemical and biological data obtained from NTA are lacking. The achievement of chemical-microbiological integrated data is of paramount importance since it may contribute to a better understanding of the anthropic pressure due to both CECs/CEC-TPs and ARB/ARGs, as well as to the implementation of better strategies for their removal in WWTPs. Furthermore, this new knowledge may guide policy makers in developing regulations and guidelines to control discharge into the environment, as well as to manage ARB/ARG risks in treated wastewater (TWW) and drinking water.

1B. __Own work and previous activities of the consortium__. The WATERPATH team is an interdisciplinary consortium with advanced competences in the fields of environmental chemistry, analytical chemistry, and environmental microbiology. The members of the consortium have already worked, individually or in mutual collaboration, on the topics of CECs/CECs-TPs, and/or ARB/ARGs occurrence in the environment. P1-UNIFI (Prof. Del Bubba) has collaborated for many years with both P2-UNITO (Prof. Bruzzoniti) and P3-ULPGC (Prof. Santana Rodriguez) in the development and application of analytical methods for the analysis of CECs in aqueous matrices [**36,37**]. P1 and P2 have also collaborated in the development and application of innovative materials (e.g., silicon carbide foams and biochar) for the removal of CECs/CEC-TPs from water

[**38,39**], which is another issue faced by the WATERPATH proposal. The collaboration between P1 and P2 also resulted in the proposition of some project proposals that were funded in the sectors of ecosystem services linked to the wastewater treatment and agricultural reuse (IRRIGATIO project and SECUREFOOD2050 project, see "Awards received/other responsibilities" in the PI-CV on the online platform). Collaborations (bilateral MIDEGRADO project and ERASMUS exchanges) are ongoing between P2 and P4 within an UNITO-BBU international agreement. P4-BBU (Prof. Beldean-Galea and co-workers) has developed over the years research focused on the analysis of antibiotics in surface waters (NATO SfP 984440) [**40**], nonsteroidal anti-inflammatory drugs, and steroids in wastewater [**41,42**], degradation by-products of NSAIDs during the Fenton process [**43**] and other applications using chromatographic-mass spectrometric techniques. P4 has recently completed a comprehensive study [RomaHealthRisk Project] focused on the quality of food and drinking water resources in Romania and the human health risk associated with the ingestion of contaminants through water and food chains [**44,45**]. P1 and P5 (Gestione Impianti Depurazione Acque S.p.A., GIDA) collaborate for many years within the UNIFI-GIDA joint laboratory "LABPUR", coordinated by the principal investigator of P1, and have published many articles – *inter alias* [**46-48**] – focusing on CECs in WWTPs managed by GIDA and in environmental waters. P3 and P1 have collaborated for a long time within an international agreement, with numerous ERASMUS researcher exchanges and scientific articles on CEC determination in tap water, wastewater, and marine organisms [**49,50**]. P1 is also collaborating with the self-funded Partner A (SFP-A, Dott. Pietro Rubellini and Dr. Fabio Cioni) in the context of the co-supervision of master's theses. As regards the microbiology component of P1 (Prof. Marvasi and Dr. Bacci), the team has a wide experience in ARB/ARGs detection [**51,52**] and computational approaches for the analysis of complex metagenomic datasets [**53-55**]. In particular P1 worked on the definition of ARGs/ARB within a new category of evolving CEC in risk assessment [**4**]. P3-ULPGC has also extensively performed its own work in the analysis of CECs/CEC-TPs in marine ecosystems [**56**]. Within the P4-BBU unit, Prof. Anca Butiuc-Keul and her team revealed the presence of bacteria carrying genes encoding resistance against antibiotics and biocides in surface waters, groundwaters, hospital effluents, influent and effluent from water treatment plants. The main scientific achievements are strongly connected with the subject of this project, which is proved by articles published about bacterial genetic resistance against antibiotics and the transfer of the resistance to other bacteria by horizontal gene transfer via integrons [**57-61**]. A wide expertise in DNA/RNA extraction and metagenomic analyses, as well as in bacteria cultivation is provided within the ULPGC research unit, by the geneticist Dr. Hyun Suk Shin and the microbiologists Prof. María T. Tejedor Junco and Dr. Margarita R. González Martín, as proven by their published literature [**62-65**].

2. Objectives, aims. Figure 1 schematically illustrates the "WATERPATH concept", i.e., the idea behind the WATERPATH proposal and how it is developed through research activities.

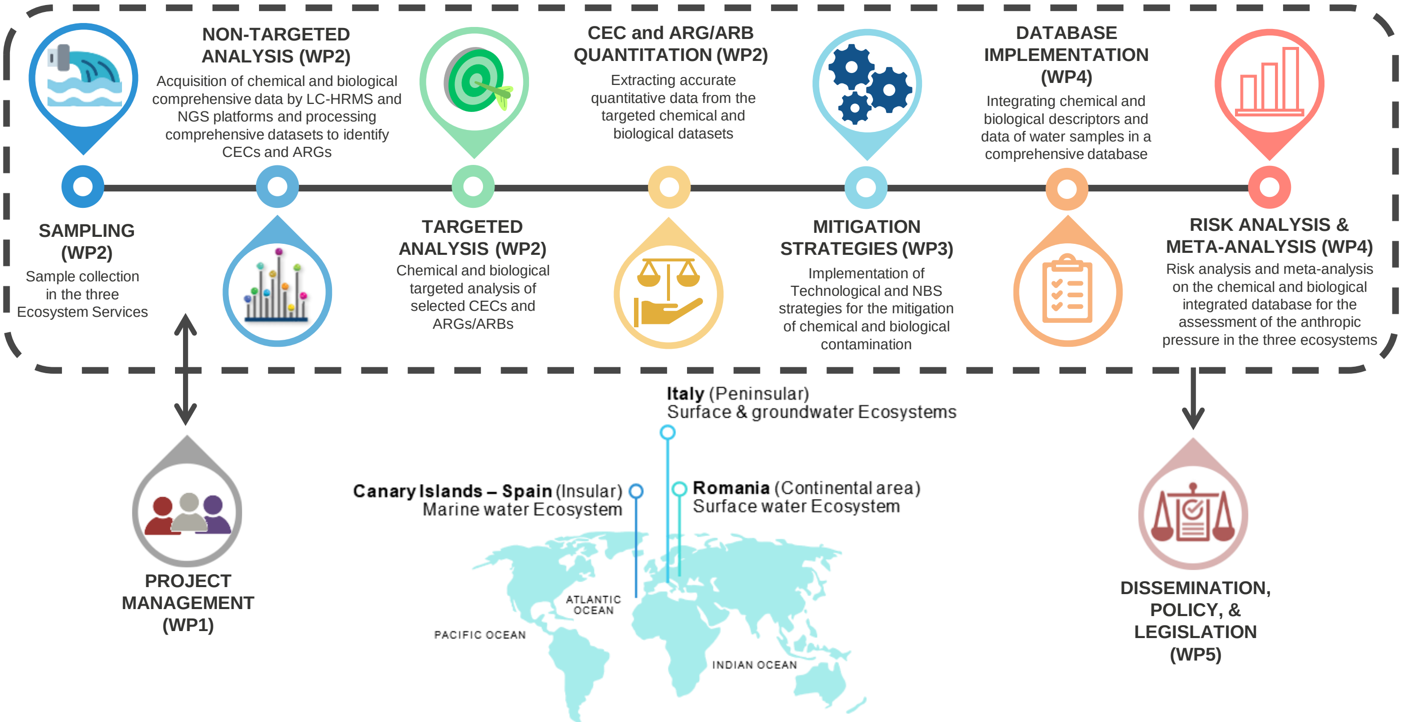

Figure 1 – The "WATERPATH concept".

WATERPATH aims to achieve the important and ambitious strategic goal of providing regulatory bodies with complete and up-to-date information on the state of chemical contamination and the effects that such contamination causes on antimicrobial resistance in three model geographical areas with complementary characteristics in terms of ecosystem services, to support the European environmental policy and in particular to contribute to the updating of the “European Priority Lists”, the “Monitoring Watch Lists” and the regional “Water Protection Plans”. WATERPATH also aims to identify mitigation strategies of chemical and biological contamination capable of offering an improvement in the ecological status of water bodies testing both high- and low-technology approaches, the latter merging nature based solutions (NBS) with the innovative concepts of waste recycling and circular economy. To contribute to the achievement of these strategic objectives, besides the academic partners, WATERPATH has included in the consortium as a self-funded partner (SFP-A) the Tuscany Environmental Protection Agency (ARPAT), an institution with both environmental control functions and regional policy support roles. Furthermore, the consortium includes GIDA, as relevant actor of the water resource management of the Tuscany region and important source of technical expertise in the field of wastewater treatment and reuse, being it involved in the treatment of wastewater from Prato (Italy) and its textile district, as well as in the reuse of TWW. WATERPATH has also contacted some other institutions and organisations operating in the geographical areas of the project proposal as stakeholders, i.e., the policy makers (i) Regione Toscana (Italy), (ii) Regione Piemonte (Italy), (iii) Consejo Insular de Aguas - Cabildo de Gran Canaria (The Canary Islands, Spain), (iv) Mureș Water Basin Administration (Romania), and the public/private companies responsible for the integrated cycle of water management (v) Publiacqua S.p.A. (Florence, Italy), (vi) Empresa Mixta de Aguas de Las Palmas (The Canary Islands, Spain), and (vii) Compania Aquaserv (Romania) that are willing to contribute to the success of the project by providing legislative support (i-iv), sharing their technical knowledge (v-vii) and being part of the Advisory Board (i-vii), as demonstrated by their signed supporting letters available upon request (see also Section 11 for more info on stakeholder’s engagement). The aforementioned general objectives will be achieved by pursuing specific objectives (SOs), which can be summarised as follows. (**SO1**) **Collect comprehensive chemical CECs/CEC-TPs data** from continental (river water, Romania), peninsular (river and ground waters, Italy) and insular (marine water, Canary Islands, Spain) ecosystem services through LC-HRMS and Py-GC-MS NTA [**66**]. These activities will be carried out by the academic partners from the three geographical areas of investigation, i.e., P1-UNIFI (Italy), P2-UNITO (Italy), P3-ULPGC (Canary Islands), and P4-BBU (Romania). Additionally, ARPAT will contribute to these activities by providing the consortium with high-level capacity building in the sampling of internal and marine waters, since it is institutionally involved in these tasks for the water chemical and biological monitoring and classification, in accordance with the Water Framework Directive. (**SO2**) **Integration of chemical qualitative data with “two-way” quantification of CECs** through (A) retrospective analysis of NTA data acquired by LC-HRMS and Py-GC-MS, as well as by (B) workflows for targeted analysis of specific CECs/CEC-TPs by LC-MS/MS and Py-GC-MS, carried out by the academic partners. (**SO3**) **Perform a risk analysis** using the Risk Quotient index for the quantified CECs/CEC-TPs (see SO2), to achieve a reliable risk assessment, associated with the chemical anthropogenic pressure on the investigated ecosystem services. (**SO4**) **Collect comprehensive and quantitative data** from the aforementioned ecosystem services (see SO1) **on ARB/ARGs** through metagenomics and targeted cultivation for the meta-analysis. (**SO5**) Identification of biomarkers to be used for assessing mitigation processes. (**SO6**) **Integration of the chemical and biological qualitative-quantitative data** obtained (see SO1-SO5), with site records on drinking, surface, ground and marine waters available by national control authorities and scientific literature to achieve CEC/CEC-TPs and ARB/ARGs spatial and temporal trends. To this aim, the consortium will take advantage from the participation of ARPAT and the organisations/institutions that provided supporting letters. Within this objective, a highly-informative database will be created as a resource for achieving the following specific objectives. (**SO7**) **Meta-analysis of chemical and biological data** obtained through NTA and targeted workflows integrated with proper qualitative descriptors (i.e., moderators), **to identify effective indexes for the evaluation of the anthropic pressure** exerted by the presence of CECs/CEC-TPs and ARB/ARGs on the investigated ecosystems, following a “one-health” approach. (**SO8**) **Evaluation of low- and high-tech mitigation strategies** as potentially suitable approaches to restore chemical and biological ecosystem quality. In more detail, two kinds of TWW refining systems will be tested for the removal of CECs/CEC-TPs and ARB/ARG biomarkers (see SO5): (i) high-tech pressure-driven nanofiltration membranes and (ii) a new concept of constructed wetland (CW) that integrates biochar (BC) produced from sewage sludge as a low-cost and sustainable filling medium for promoting long-term removal of CECs/CEC-TPs and eventually ARB/ARGs, thus realising a low-tech NBS combined with a circular and virtuous waste management strategy.

3. Relevance to the call (including theme(s)). WATERPATH covers the Topic 2 of the call, i.e., “Understanding and predicting multiple pressures (including anthropogenic pressures) – impact – response relationships in ecosystem services through advanced methods and techniques” with particular reference to the assessment of the effect of different anthropogenic pressures from human activities and cumulative effects on ecosystems and ecosystem services (sub-topic 2.1), as well as to the development of innovative approaches for the restoration of aquatic systems and ecosystem services (sub-topic 2.3). Furthermore, WATERPATH is involved in Topic 1 "Mapping, monitoring, and assessment for a better understanding of ecosystem services in a context of changes, from local to global change", particularly by promoting the integration of previously existing and WATERPATH data sets through a meta-analysis across temporal and spatial scales (sub-topic 1.1).

4. Concept, methods. This section reports the methods used by the WATERPATH consortium to carry out the work plan described in Section 8 of the project proposal. The methodology followed for Project Management (WP1) will be based on both online and in-person meetings. Continuous check of the correct progress of all project activities will be ensured through online meetings organised monthly, involving the whole boards described in Section 9 of the project proposal or some of their members, depending on the needs required by the timing of the project. General Assembly Meetings (GAMs) will be organised in presence, at the beginning, in the middle, and at the end of the project, according to a rotation scheme of the host venue. During the first GAM, the organisation and decision-making structure of WATERPATH will be explained with full details by PC to all participants and the Project Boards will be formally constituted. Project management will also cover the issue of intellectual property protection, which will be fully defined in the consortium agreement (Task 1.1). In particular, intellectual property rights (IPR) will be considered in order to negotiate any relevant questions with partners before starting the project, according to a fairness principle to retain intellectual property rights according to partners contributions. The pre-existing knowledge that each partner makes available to the the consortium for carrying out the project will be detailed in the consortium agreement. However, no particular barrier to sharing materials or results is foreseen, thus following the general "Open Science" approach. Sampling (Task 2.1). Ecosystem services under investigation will be located in Central and North Italy (i.e., Tuscany and Piedmont regions), Gran Canaria Island (Spain), and Romania, thus collecting a wide spectrum of environmental scenarios, including differences in water services (e.g., potabilization and depuration) and in WWTP treatment technologies. According to the established consortium agreement and the Project Management Handbook (PMH, see also Section 8), the consortium will adopt homogeneous time-scheduled sampling protocols, including storage for chemical/biological analysis, to obtain comparability within the collected samples. To properly evaluate CEC/CEC-TP, and ARB/ARG pressures, samples will include raw waters before potabilization treatment, potable water, WWTP effluents, river water upstream and at different distances downstream WWTP discharge, sea water at the WWTP discharge and internal and marine water samples, including also ground and river waters intended for agricultural irrigation, chosen on the basis of previous experience of the consortium, as well as institutional organisation records. The collection of water samples will be carried out using refrigerated sampling stations by taking average samples over 24 hours (approximately 9-10 L). Grab sampling will be considered only in exceptional situations, when strictly necessary. Samples for the Tasks 2.3 and 2.5 will be collected using plastic-free equipment. Samples will be stored at -20°C until analysis. Chemical NTA (Task 2.2). To encompass the detectable chemical coverage by the NTA methods, the acquisition protocols will be optimised by employing a large training set of chemically heterogeneous CECs and CEC-TPs, including those listed in most recent European watch list of chemical substances (e.g., Decision 2022/1307) and prioritising lists available in the scientific community (e.g., compounds listed in USEPA ENTACT mixtures and NORMAN SLE databases). This objective will be pursued by means of the Design of Experiment (DoE) approach, which by its multivariate nature, allows for simultaneously optimising both the chromatographic behaviour and MS responses of CEC/CEC-TP training set [**22**]. Additionally, depending on the desired sensitivity and sample volumes to be analysed, NTA workflows will be implemented with sample clean-up/enrichment protocols (e.g., offline or online solid phase extraction) to increase the probability of CEC and CEC-TP detection and to simultaneously reduce the MS signal suppression/amplification (i.e., not negligible matrix effects). In parallel, customised MS/MS experiments in data-dependent analysis fashion will be carried out on the investigated samples to obtain useful structural insights on the detected CECs/CEC-TPs (e.g., suspect screening lists). After the optimization and analysis phases, the acquired data will be processed using prioritisation workflows for feature (i.e., precursor ion described by m/z and retention time) detection based on signal intensity and/or suspect lists. Level I or level II feature identifications will be provided by measuring reference standards under the same experimental conditions of sample analysis or using surrogate standards chosen based on t_R data, mass spectra, and response factors, respectively. Microplastics screening (Task 2.3). Microplastic extraction from water samples will be performed by means of stainless steel filters, after density separation and oxidation procedures, avoiding the use of plastic equipment. After extraction, the residual particles will be preliminarily characterised by visual observation using a Nikon H550S stereomicroscope equipped with Micro Capture Ver 6.9.12 software (DIV = 0.01 mm). In order to reduce subjectivity and random errors, fragments will be classified into morphology, optical property and behaviour categories, according to the flow chart suggested elsewhere [**67**]. The PMH will ensure comprehensive and homogeneous data by outlining NTA/screening method criteria selection for all the three investigated geographical areas, essential for the next phases of risk analysis and meta-analysis. Targeted chemical analysis of CECs and CEC-TPs (Task 2.4). Once CEC and CEC-TP lists are obtained from Task 2.1, targeted MS/MS analysis workflows based on multiple reaction monitoring acquisition strategies, both in high and low resolution mode (LC-HR-MRM and LC-MRM), will be carried out to quantitatively assess CEC/CEC-TP occurrence in the collected water samples. For CECs and CEC-TPs with available reference analytical standards, quantification protocols based on external and/or matrix matched calibration curves will be used. Conversely, in case of unavailability of analytical standards, a semi-quantitative procedure (e.g., quantification by surrogate standard) will be performed with respect to physicochemical similar standards (e.g., same chemical class and structural homologues) to the CECs/CEC-TPs of interest [**16**]. As defined for chemical NTA workflows, methods' sensitivity will be adjusted implementing large volume direct injection or enrichment by solid phase extraction for the investigated samples. Microplastic quantitation (Task 2.5). Once classified, microplastics will be further analysed through Py-GC-MS to confirm their nature and to quantify polymer occurrence in water samples. Also within this task, in order to obtain highly reproducible datasets among the partners involved in the different geographical areas, the PMH will guide the consortium in the selection of the proper quantitative workflows, to scale-up CEC quantification according to the needs of each investigated matrix (e.g., sensitivity and matrix effect). Biological Analyses (Task 2.6). High-quality DNA will be extracted by using commercial kits, and subsequently, libraries for NGS will be prepared. Metagenomic NTA approaches, using NGS, will be employed to identify the resistome and profile of the microorganisms associated with the detected ARGs. The choice of NGS platform, such as Illumina or PacBio, will be made at the time of sequencing to accommodate the rapid advancements in bioinformatics and hardware technologies. To give support to the resistome profiles linked to selected bacterial taxonomic groups, we will employ cultivation methods. The determination of target genes and bacteria will depend on the specificity of available media. On isolates, the characterization of resistant colonies will be completed by 16S (for the identification at the genus level) and sampling sequencing. The analysis will encompass: (i) Penicillins: Testing susceptibility to Ampicillin (AMP) and Piperacillin-tazobactam (TZP), with a focus on ARGs such as AMP, TEM-1, TEM-2, SHV-1, CTX-M, and PER-1; (ii) Cephalosporins: Assessing resistance using Ceftazidime (CFZ) and Cefepime (CPM), with attention to AmpC ARGs; (iii) Carbapenems: Using Imipenem (IMI) to evaluate susceptibility, while screening for ARGs including KPC, VIM-1, VIM-2, NDM-1, OXA-48, and PstS; (iv) Quinolones: Checking the efficacy of Ciprofloxacin (CIP) and Norfloxacin (NOR), with qnrA, qnrB, and qnrS ARGs as indicators. (v) Aminoglycosides: Testing Gentamicin (GEN) effectiveness, and investigating ARGs such as aac(3')-I, aac(6')-Im, aac(6')-II, aph(2')-Ib, among others; (vi) Glycopeptides: Only in Enterococcus, testing for Vancomycin (VAN) susceptibility and detecting VanA and VanB ARGs; (vii) Macrolides: Evaluating Erythromycin (ERY) susceptibility and identifying ARGs including ermA, ermB, ermC, mefA, and msrA; (viii) Tetracyclines: Determining the resistance against Tetracycline (TET) and identifying ARGs such as tetA, tetB, tetC, tetK, tetL, and tetM; (ix) Trimethoprim-sulfamethoxazole: Testing Trimethoprim-sulfamethoxazole (SXT) effectiveness, with ARGs sul1, sul2, and sul3 in focus. In summary, the plan will include testing a total of 12 antibiotics for Enterobacteriaceae, 10 for Pseudomonas, and 8 for Enterococcus. A range of 40 ARGs will be assessed for Enterobacteriaceae, 27 for Pseudomonas, and 21 for Enterococcus. This approach will allow us to rank the critical ARGs and their hosts. Mitigation strategies (Tasks 3.1, 3.2, and 3.3). The vertical and horizontal CWs will be designed based on the expertise previously acquired within the project coordinator's unit [**68,69**], managing the CWs in order to test competitive sizing compared to current literature (e.g., < 1 m^2 per population equivalent (p.e.) per day). CWs will be implemented as lab-scale systems (1-2 m^2 of surface area) filling the beds with high surface area granular biochar (SSA > 350 m^2/g) obtained by pyrolysis of sewage sludge at 850°C for 2 h, followed by an acidic washing and final thermal activation at 650°C for 1h [**68**]. Commercially available high-tech pressure-driven nanofiltration membrane systems (NMS) with pore size in the range of about 0.2-2 nm, in principle suitable to exclude CECs/CEC-TPs from permeate, will be tested. Both membranes functionalized with anionic and cationic exchangers will be used to highlight eventual differences in the removal of basic and acidic pollutants. The NMSs and CWs will be fed by TWWs and monitored for the removal of CECs/CEC-TPs and ARB/ARGs (i.e., biomarkers), according to the aforementioned target approaches. Risk Analysis (Tasks 4.2 and 4.3). Regarding the human health risk, in the case of non-carcinogenic compounds, risk assessment will be performed using the Risk Quotient index, calculated dividing the minimum and the maximum Measured Environmental Concentrations (MECs) by a reference dose (RfD), thus evaluating the risk in the best- and worst-case scenarios. RfD will be calculated dividing the non-observed adverse effect level (NOAEL) by an appropriate assessment factor, ranging between 100 and 10000, depending on the compound considered. Finally, a daily intake dose is calculated based on concentration in drinking water, considering both adult and child exposure according to the USEPA guidance [36]. For the eventual occurrence of carcinogenic compounds, the risk will be calculated by multiplying the daily intake for the slope factor derived from the Integrated Risk Information System (IRIS) EPA database. For the environmental risk analysis, the same procedure of non-carcinogenic contaminants is adopted, considering No-Observed Effect Concentration (NOEC), or LC50/EC50 data instead of NOAEL. Appropriate assessment factors will be chosen according to the ECHA guidance on short- and long-term toxicity results, also considering the most sensitive reference trophic levels among fish, aquatic invertebrates, and algae. In detail, the procedure described below, widely adopted in literature [**70**], will be followed. RfD will be calculated dividing the minimum and the maximum Measured Environmental Concentrations (MECs) by the Predicted No-Effect Concentration (PNEC), thus evaluating the risk in both scenarios. Predicted no-effect concentration (PNEC) will be calculated dividing the No-Observed Effect Concentration (NOEC) by an appropriate assessment factor, chosen according to the ECHA guidance on short- and long-term toxicity results. In the case that no NOEC values are available, LC50 or EC50 values will be used instead. Fish, aquatic invertebrates, and algae will be used as reference trophic levels, considering the most sensitive (i.e., the one with the lowest NOEC/LC50/EC50 value) for risk assessment. In particular, if an acute toxicity value for at least one trophic level is available, the assessment factor shall be considered as a precautionary measure of 1000. In the case that a chronic NOEC value of either fish or aquatic invertebrates exists, an assessment factor of 100 will be used. Finally, when two or three chronic NOEC values will be available assessment factors of 50 and 10 will be used, respectively. RQ values less than 1 will indicate that the CEC is less likely to pose a considerable risk to the environment. <u>Meta-analysis (Task 4.4)</u>. Meta-analysis is used to summarise the pressure exerted on the ecosystem services, investigating how chemical and biological quali-quantitative data impact on their quality, following a “one-health” approach. To this extent, the database obtained in Task 4.1 will be processed to organise the experimental data and related meta-data on the identified and quantified CECs/CEC-TPs and ARBs/ARGs into moderators for meta-analysis (i.e., a chemical and biological intra-database). Chemical or biological observations will be categorised according to proper methodological *moderators*, describing for instance, their origin (literature or experimental data), year of collection, variable type and level (e.g., chemical or biological observation and category), number of observations, and available quantitative data (e.g., concentrations found, mean values, and measures of variability ($n > 2$)). For each observation, ecosystem pressure will be described by response ratios (RRs) of relevant chemical (e.g., CECs concentrations) and biological (e.g., bacteria and genes) effects. In detail, the size of effects will be determined by calculating RRs of the impacted (i.e., experimental) conditions vs. control conditions (i.e., limits of detection/ quantitation or relative quantification to control genes), and will be reported as a quantitative stress index in the intra-database for each variable [**71**]. The size of the effects on ecosystem services will be determined by generalised linear mixed models (GLMMs), including the random effects (e.g., sampling sites) related to the study design. According to the significance of the obtained models, the quantitative balance of anthropic pressure will be outlined (i.e., RR plots) to point out the ecosystem service stress and quality. <u>Capacity building (Task 5.2)</u> will be carried out by promoting exchange programmes of young researchers among the labs of the consortium’s partners, as well as by implementing online courses on specific topics faced by the WATERPATH project. <u>Divulgation to the Scientific Community (Task 5.3)</u> will be performed through open access scientific articles published in international peer-reviewed journals, as well as by means of contributions in national and international conferences. <u>Divulgation to the general public (Task 5.4)</u> will be carried out by communicating to the social community the most relevant results of WATERPATH, using social media, in person and/or online meetings, as well as round tables open to the public. <u>Divulgation to stakeholders (Task 5.5)</u> will be performed by involving the final recipients of WATERPATH activities (e.g., companies involved in the integrated management of the water cycle) in meetings and visits to the demonstration sites for explaining the environmental, economic and social impacts achieved by the project. Based on the results obtained for the various research activities of the consortium (i.e. monitoring of ecosystems, obtaining innovative materials for wastewater treatment and their application for the mitigation of ecosystem pollution, innovative strategies for the evaluation of anthropic pressure on ecosystem services), WATERPATH will contribute to <u>Policy and Legislation (Task 5.6)</u> by examining the existing strategies, policies and legislation in the aforementioned fields of investigation, also with the help of various political actors who have expressed an interest in collaborating with the consortium (supporting letters available upon request). A policy brief will then be prepared with the aim of strengthening the political, institutional and regulatory framework for the aforementioned sectors.

5. Explanation of the novelty of the research planned, in relation to the present state-of-the-art. WATERPATH proposal is characterised by the following novelties in relation to the current state-of-the-art. (i) An innovative approach in the optimization of instrumental parameters of chemical NTA, based on the implementation of a training group of analytes and a multivariate DoE analysis. (ii) The comprehensive study, by means of homogeneous methods, of the presence of CECs/CEC-TPs in continental, peninsular and island ecosystems, thus providing new information, overall characterised by a high complementarity level. (iii) The comprehensive study, by means of homogeneous methods, of the presence of ARB/ARGs in the three types of ecosystems, providing new and highly complementary information also from the biological point of view, including the possible identification of biomarkers of the investigated areas. (iv) The simultaneous and systematic study in these three geographical areas of crucial ecosystem services linking WWTP effluents, water bodies receiving these effluents, and water supply for irrigation and drinking purposes from both the chemical and biological points of view. (v) The study of wastewater refining systems, including innovative and sustainable nature-based approaches, aimed at mitigating the impact of selected CECs/CEC-TPs and ARB/ARGs (i.e., biomarkers) in water bodies that are part of ecosystem services dedicated to the production of drinking and irrigation waters. (vi) The integration of chemical and biological data in a unique and comprehensive database, which includes information on the temporal and spatial scales on the presence of CECs/CEC-TPs and ARB/ARGs as moderators of anthropogenic pressure, thus providing through a meta-analysis approach an intra-database capable of describing the extent of chemical and biological pressure exerted on ecosystems.

6. Expected results and how they lead to impact. Below are the main impacts generated by the results of WATERPATH (references to the impacts of the call are in bold). (i) The WATERPATH consortium pursues the objective to provide large-scale quali-quantitative information on the pressure of CECs on key ecosystem services in geographically distinct but globally representative areas, thus providing relevant information for **improving ecosystem governance for the provision and protection of related ecosystem services** to policy makers within the consortium geographical areas. The WATERPATH consortium, in fact, includes policy makers (i.e., Regione Toscana and Regione Piemonte), as well as institutions acting as regulatory support (i.e., ARPAT, Consejo Insular de Aguas de Gran Canaria, and Mureș Water Basin Administration), as partners or parties that have signed letters of interest. (ii) It is remarkable that, in each geographical area, WATERPATH will provide comprehensive data on different ecosystems and ecosystem services pursuing a concept of **integration of ecosystem services approaches.** The acronym WATERPATH, in fact, recalls the idea behind this proposal, namely the observation of the quality of water during its "journey" through different ecosystems and ecosystem services (e.g., from WWTP to the sea and from the sea to the potabilization plant or from WWTP to the river and from the river to the agricultural irrigation). (iii) The accurate knowledge of CECs/CEC-TPs and ARB/ARGs in the ecosystems studied undoubtedly represents the first and essential step to understand which technical and political strategies must be adopted to alleviate anthropogenic pressure on these ecosystems, thus contributing to the **restoration of natural ecosystems for the conservation of ecosystem services.** For example, according to EU Directive 2020/2184, Member States shall ensure that the ecosystem service which involves supply, treatment and distribution of water intended for human consumption is subject to a risk-based approach that covers the whole drinking water supply chain. Hence, the water suppliers shall carry out a risk assessment of the supply system for the first time by 12 January 2029. In this regard, the WATERPATH project will support water suppliers and policy makers with this new task for which it is necessary to develop new competencies. Similar considerations regarding the adoption of the risk-based approach can be made for the reuse of TWW according to the EU Regulation 2020/741. (iv) WATERPATH intends to prepare a policy brief in accessible language that (a) contains the description of current policies, (b) summarises the scientific evidence derived from the project, and (c) proposes recommendations on future policies, thus contributing to an **improved governance of ecosystems for the delivery and protection of their ecosystem services**. (v) Moreover, WATERPATH will contribute directly to the **restoration of natural ecosystems for the conservation of ecosystem services** by testing both high-technology mitigation techniques and innovative concepts of CWs as NBS for the removal of chemical CECs/CEC-TPs and ARB/ARGs from the effluents of WWTPs in the three geographical areas covered by the proposal. (vi) Last but not least, the results obtained from WATERPATH will have a significant impact on the **UN SDGs** 3 (Good health and well-being) and 6 (clear water and sanitation) and are in line with the **EU's Green Deal** and **Biodiversity Strategy** for 2030, which represents a comprehensive, ambitious and long-term plan to protect nature and reverse the degradation of ecosystems.

7. Transnational added value of the research proposed. WATERPATH considers three model geographical areas (i.e., continental, peninsular and insular) with complementary features in terms of suffered anthropic pressure (i.e., industrial, agricultural and tourist impacts) and ecosystem services under investigation (for example, drinking water from groundwater, river or sea water), thus providing results that go beyond the specific locations investigated and can be transferred to other environmental scenarios and related ecosystem services. WATERPATH intends to generate a critical mass of results that overall lead to obtaining solutions to common problems, in a fully transnational perspective. This information will be made available to various international stakeholders through an appropriate Plan of Dissemination, Exploitation and Communication of the results obtained (DECP), thus generating transnational learning opportunities (see WP5). In this framework, WATERPATH DECP will implement demonstration actions, including audience readable outcomes (e.g., intra-databases) in public repositories and evaluate the impact of proposed mitigation strategies (WP3) in improving water-based ecosystem services. It should also be noted that the WATERPATH Consortium is assisted by local authorities and companies of wastewater and drinking water management sectors in the three countries, which have already signed supporting letters, thus creating the conditions for obtaining not only reliable scientific data, but also their effective transfer to the European society, the scientific community, and policymakers. All these actions will result in a high level of transnational cooperation within the consortium and beyond. Last but not least WATERPATH intends to pursue transnational added value through the collaboration with ongoing international initiatives regarding CEC/CEC-TP and/or ARB/ARG spreading in ecosystems, such as the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR), which involves 19 European countries with the main goal of developing "integrated approaches to pursue unique world-class research on AMR that will be translated into new prevention and intervention strategies that improve the public health and wellbeing of populations, and delivers economic and societal benefit throughout Europe and beyond".

8. Workplan. The work plan is organised in the following five work packages (WPs), including specific deliverables that, in agreement with the WATER4ALL call announcement, are intended as tools for monitoring the progress and the complete achievement of the project objectives. Months and deliverables are abbreviated as "*M*" and "*D*", respectively. The partners of the consortium are abbreviated as specified: (i) "P1" - Project Coordinator University of Florence (UNIFI), Italy; (ii) "P2" - University of Turin (UNITO), Italy; "P3" - University of Las Palmas de Gran Canaria (ULPGC), Spain; (iv) "P4" - Babeș-Bolyai University (BBU), Romania; (v) "P5" - G.I.D.A. S.p.A. (GIDA), Italy; (vi) "SFP-A" - Environmental Protection Agency of Tuscany (ARPAT), Italy.

WP1. Project Management. (WP Leader: P1 – Massimo Del Bubba). This WP aims to ensure strategic control of technical WPs, granting accordance with the timing programmed in the Gantt chart, and adherence to the governance structure of the project (see Section 9) that will be fully detailed in the "Project Management Handbook" (PMH). Task 1.1 – Consortium agreement (Task Leader: P1 – Massimo Del Bubba; Task Participants: all Partners; *M1-3*). A consortium agreement will be prepared within the first 3 months of the project. Task 1.2 – Management and harmonisation of the consortium activities (Task Leaders: P1 – Massimo Del Bubba and Massimiliano Marvasi; Task Participants: all Partners; *M1-36*). An electronic "Project Management Handbook" (PMH) in the form of an in-cloud repository with different access levels (e.g., administrator, WATERPATH users, and external users) will be prepared within the first three months of the project and updated during the project, as illustrated in the Gantt chart (see Section 10 of the WATERPATH Proposal). In its first version, the PMH will be organised in the following topics: (i) WATERPATH key appointments and related contents, including but not limited to kick-off, mid-term, and final meetings of the project; (ii) composition and acting roles of the WATERPATH boards; (iii) harmonised technical and experimental protocols; (iv) harmonised protocols for data management (Data Management Plan, DMP); (v) harmonised risk mitigation strategies. For each topic, deliverables will be provided in the form of electronic reports, and included in the first and updated versions of PMH. Deliverables. D.1.1.1 – Consortium Agreement (*M3*); D.1.2.1 – Project Management Handbook (*M3*); D.1.2.2 – First PMH update (*M9*); D.1.2.3 – Second PMH update (*M15*); D.1.2.4 – Third PMH update (*M21*); D.1.2.5 – Fourth PMH update (*M27*); D.1.2.6 – Fifth PMH update (*M36*).

WP2. Spatial and temporal monitoring of chemical and biological contamination (CECs/CEC-TPs and ARB/ARGs). (WP Leader: P3 – José Juan Santana Rodriguez). Task 2.1 – Water sampling (Task Leader: P3 – Sarah Montesdeoca Esponda; Task Participants: P1, P2, P3, P4, P5, SFP-A; *M4-24*). The sampling activities will regard the collection of water samples for the analysis described in Tasks 2.2-2.6. CECs will be studied in the following relevant ecosystem services: (i) three river basins (Po and Arno in Italy and Mures in Romania), including in the monitoring the points of water intake for the production of drinking water, the points of discharge of effluents from WWTPs, and the points upstream and downstream of these discharges, as well as a series of other points representing the collection of water for irrigation purposes; (ii) the underground water basin in the Pistoia area (Italy) used for the production of drinking water and for irrigation; (iii) three marine coastal areas, with different locations, characteristics, and anthropogenic pollution sources, i.e., Las Canteras, Arinaga, and Playa del Inglés, located on the northeast, the southeast, and the south coasts of the Gran Canaria Island, respectively, which represent areas where the three WWTPs of the island discharge their TWWs, but also zones of production of potable water by desalination. The monitoring of the aforementioned sampling point for NTA will be performed on a four-monthly time scale for the first two years of project (i.e., six samplings), thus obtaining both a spatial and a temporal picture of CEC/CEC-TP occurrence (see Section 4 for further details). Task 2.2 – NTA of chemical CECs/CEC-TPs by LC-HRMS (Task Leader: P3 – Rayco Guedes Alonso; Task Participants: P1, P2, P3, P4, P5, SFP-A; *M4-26*). Samples collected as explained in Section 4 will be processed for NTA by (i) LC-HRMS full scan experiments followed by (ii) tandem high-resolution mass experiments (LC-MS/HRMS) for structural elucidation, and (iii) level I or level II identification (see Section 4 for further details). NTA protocols will be established among partners in order to achieve common analytical procedures and high data quality. Task 2.3 – Microplastics screening (Task Leader: P2 – Luca Rivoira; Task Participants: P1, P3, P4, P5, SFP-A; *M4-26*). Samples collected in Task 2.1 will be pre-treated by the optimal combination of density separation and oxidation protocols in order to extract microplastics and separate them from interferences. The extracted fractions will be firstly characterised by visual observation using a stereomicroscope, classifying fragments according to morphology, optical properties, and mechanical behaviour; further confirmation will be performed by Py-GC-MS. Task 2.4 – Targeted analysis of CECs/CEC-TPs by LC-MS/MS (Task Leader: P4 – Mihail Simion Beldean-Galea; Task Participants: P1, P3, P4, P5, SFP-A; *M7-29*). Once CEC/CEC-TP lists are obtained, targeted MS/MS analysis workflows based on both LC-HR-MRM and LC-MRM acquisition strategies, will be carried out to quantitatively assess CEC occurrence in the selected water samples. For CECs with available reference analytical standards, quantification protocols based on external and/or matrix matched calibration curves will be used. Conversely, in case of analytical standard unavailability, a surrogate-standard semi-quantitative procedure will be performed [**16**]. Even within this task, protocols for the selection of quantitative methods will be defined, to scale-up CEC quantification according to the needs of each investigated matrix (e.g., sensitivity and matrix effect). Task 2.5 – Target analysis of microplastics by Py-GC-MS (Task Leader P2 – Luca Rivoira; Task Participants: P1, P3, P4, P5, SFP-A; *M7-29*). For each polymer identified in Task 2.3, most specific pyrolysis peaks and characteristic ions will be selected. The obtained quantitation ions will be finally used to build an external calibration curve using microplastic reference standards for each polymer to quantitatively assess microplastic occurrence in the selected water samples. Task 2.6 - NTA of resistome and microbial communities (Task Leader P1 – Giovanni Bacci; Task Participants: P1, P3, P4, P5; *M7-27*). ARGs and related microorganisms present in aquatic environments will be screened by the NGS approach using Illumina and/or PacBio technologies. Data will be stored in dedicated repositories and used for the chemical-biological mutual data integration by meta-analysis (Task 4.4). Task 2.7 – Targeted approach of cultivable ARB (Task Leader P4 – Anca Keul; Task Participants: P1, P3, P4, P5; *M7-27*). Antimicrobial susceptibility testing and bacterial 16S rDNA will be isolated from the same water samples to support and confirm the results from the NTA of resistome. A cultivable approach will be used to grow microorganisms on different antibiotic selective pressures and to identify isolates at the genus level by the 16S rDNA gene. Deliverables. D.2.2.1 – Reports on NTA (*M6,M10,M14,M18,M22,M26*); D.2.3.1 – Report on microplastics screening (*M6,M10,M14,M18,M22,M26*); D.2.4.1 – Report on CEC targeted analysis (*M9,M13,M17,M21,M25,M29*); D.2.5.1 – Report on targeted microplastics analysis (*M9,M13,M17,M21,M25,M29*); D.2.6.1 – Report on biological NTA (*M9,M13,M17,M21,M25,M29*); D.2.7.1 – Report on biological target analyses (*M9,M13,M17,M21,M25,M29*).

WP3 – Mitigation strategies for improving water-based ecosystem services (WP Leader: P5). Task 3.1 – Design and implementation of biochar-based CWs (Task Leader: P1 – Massimo Del Bubba; Task Participants: P2, P3, P4, P5; *M6-12*). Vertical and/or horizontal lab-scale flow CWs will be designed and implemented for the refining of TWW from the WWTP of the city of Prato (Italy), based on data supplied by P5. The CWs will be implemented following an innovative strategy, i.e. (i) using biochar produced by pyrolytic thermal conversion of sewage sludge as the sole filling medium of the treatment system, to promote the adsorption-based removal of CECs/CEC-TPs and ARB/ARGs, and (ii) adopting a photovoltaic artificial aeration system to increase their aerobic degradation. Task 3.2 – Design and implementation of nanofiltration membrane systems (NMSs) (Task Leader: P5 – Roberto Camisa; Task Participants: P2, P3, P4, P5; *M6-12*). Lab-scale refining systems based on nanofiltration technology will be implemented for the dimensional exclusion and/or ionic exchange removal of CECs/CEC-TPs and ARB/ARGs. Membranes made of different polymers, functionalization, and pore size will be tested. Task 3.3 – Evaluation of the removal efficiency of CWs and NMSs (Task Leader: P1 – Massimo Del Bubba; Task Participants: P2, P3, P4, P5; *M12-30*). The removal efficiency of CWs and NMSs will be monitored for CECs/CEC-TPs and ARB/ARGs (biomarkers) detected in WP2. Deliverables. D.3.1.1 – Report on design and implementation of CWs (*M*12); D.3.2.1 – Report on design and implementation of NMSs (*M*12); D.3.3.1 – Report on removal efficiency of CWs and NMSs (*M*30).

WP4 – Risk evaluation and meta-analysis. **(WP Leader: P2).** Task 4.1 – Chemical and biological database implementation (Task Leader: P1 – Massimiliano Marvasi; Task Participants: all partners; *M25-28*). The cumulative results of WP2 will consist of qualitative-quantitative datasets containing the main physicochemical descriptors and quantitative data of CECs/CEC-TPs and ARB/ARGs occurring in the selected water samples. According to FAIR guidelines, the experimental data will be supported by meta-data (e.g., method and acquisition mode, reference standard, and sample type), fundamental for the correct contextualization of the risk for the investigated ecosystem services (see Tasks 4.2 and 4.3) and the identification of an intra-database quantitative index describing the anthropic pressure of the identified and quantified CECs/CEC-TPs and ARB/ARGs (see Task 4.4). Task 4.2 – Risk-based approach to environmental water safety (Task Leader: P4 – Mihail Simion Beldean-Galea; Task Participants: all partners; *M29-34*). For each CEC, risk assessment will be performed using the Risk Quotient index, as specified in the Method section. Task 4.3 – Risk-based approach to drinking water safety (Task Leader: P2 – Maria Concetta Bruzzoniti; Task Participants: all partners; *M29-34*). Toxicity assessment will be performed for both carcinogenic and non-carcinogenic risk, using slope factors and RfD, respectively, derived from the IRIS EPA database, as specified in the Method section. Task 4.4 – Meta-analysis (Task Leaders: P1 – Massimo Del Bubba and Massimiliano Marvasi; Task Participants: all partners; *M29-M34*). The extraction of qualitative-

quantitative data from the database implemented within Task 4.1 will be used for performing a meta-analysis aiming at summarising the wide and complex information generated in WP2 in an intra-database quantitative index describing the anthropic pressure of the identified and quantified CECs/CEC-TPs and ARB/ARGs. Deliverables. D.4.1.1 – Database implementation (M28); D.4.2.1 – Report on environmental risk analysis (M34); D.4.3.1 – Report on risk analysis for drinking water (M34); D.4.4.1 – Report on meta-analysis (M34).
WP5 – Communication, Dissemination and Exploitation of Results (WP Leader: P4 – Mihail Simion Beldean-Galea). Dissemination will be delivered to the scientific community, policy makers, stakeholders, and general public. Qualitative and quantitative criteria will be used for assessing the impact of project communication activities (see also "Exploitation and dissemination of results" section). Task 5.1 – Web page (Task Leader: P2 – Maria Concetta Bruzzoniti; Task Participants: all partners; *M1-36*). A web-page will be prepared within two months after project start and updated regularly. Task 5.2 – Capacity building (Task Leader: SFP-A – Fabio Cioni; Task Participants: all partners; *M12-36*)**.** A capacity building strategy will be designed and included in the PMH. Task 5.3 – Divulgation to the Scientific Community (Task Leader: P3 – Margarita Rosa Gonzalez Martin; Task Participants: all partners; *M10-36*). This task includes presentations of results at conferences, publication of articles in international peer reviewed journals and the organisation of a final International Symposium on the project themes. Task 5.4 – Divulgation to the general public (Task Leader: P4 – Vlad Alexandru Panescu; Task Participants: all partners; *M6-36*). This task focuses on the diffusion of information through social networks, radio and TV interviews, round-tables and public divulgation meetings. Task 5.5. – Divulgation to stakeholders in the field of wastewater treatment (Task Leaders: P5 – Donatella Fibbi and Ester Coppini; Task Participants: all partners; *M12-36*). This task will operate through on-field demonstration activities and workshops with companies involved in wastewater treatment to disseminate the technologies tested within WATERPATH, thus following a participatory approach. Task 5.6 – Policy and legislation (Task leader: SFP-A – Pietro Rubellini; Task Participants: all partners; *M12-36*). This task aims to inform political decision makers about WATERPATH results, supporting environmental policy actions and update of the European community's priority and monitoring lists. This task aims also to inform the actors of the political decision-making process of the results related to the recycling of biological sludge for the production of biochar, contributing to the end-of-waste process of this waste, according to the recent European Waste Framework Directive 2018/851, which defines the end-of-waste legal tool in a circular economy perspective. A policy brief will be drafted with the assistance of political actors who have signed letters of support. Deliverables. D.5.1.1 – Web page (*M2,M12,M24,M36*); D.5.2.1 – Report on capacity building (*M24,M36*); D.5.3.1 – Report on dissemination to the Scientific Community (*M18,M36*); D.5.4.1 – Report on dissemination to the general public (*M12,M24,M36*); D.5.5.1 – Report on dissemination to stakeholders (*M24,M36*); D.5.6.1 – Report on contributions to policy and legislation (policy brief) (*M24,M36*).
9. Project coordination and management. The WATERPATH governance structure is schematically illustrated in **Figure 2**.

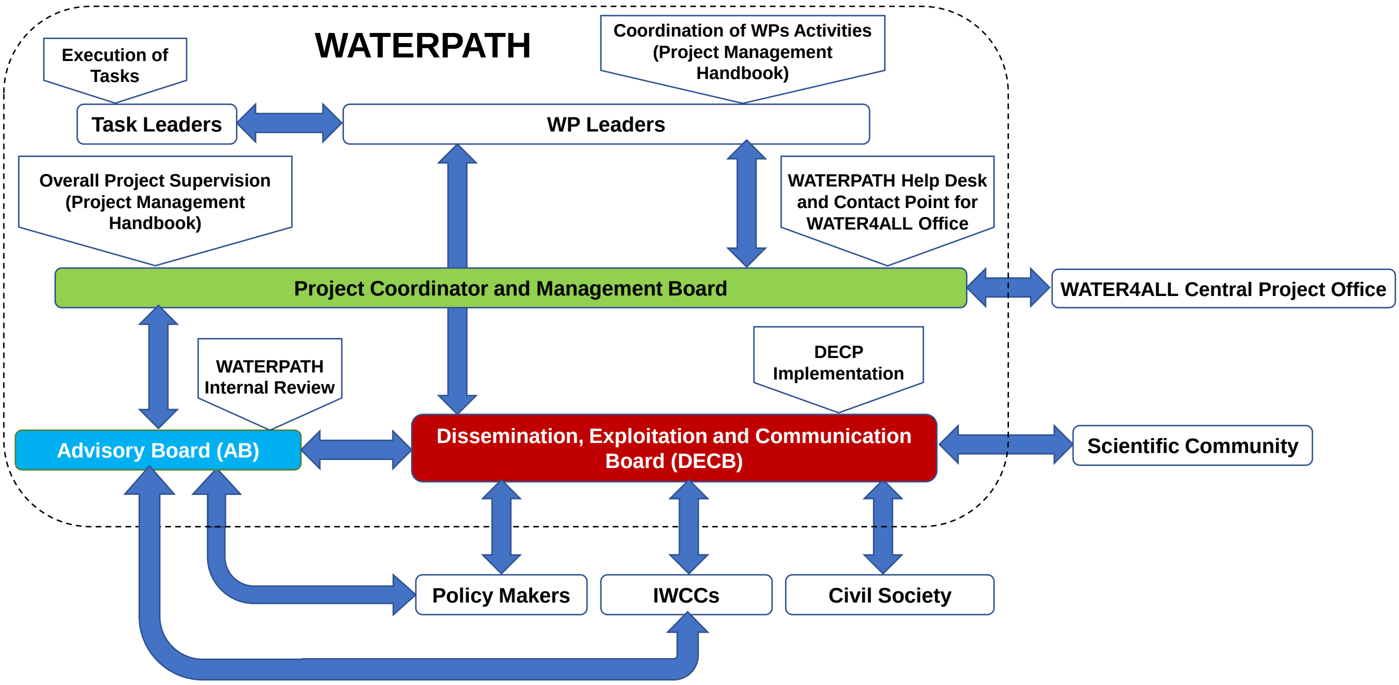

Figure 2 – Schematic illustration of the WATERPATH governance. Note that companies involved in the integrated cycle of water management are abbreviated as IWCCs.

This structure is designed in order to guarantee an equivalent representation of each partner in the various boards. Moreover, gender balance in the research teams and in decision-making will be fostered. The **Management Board (MB)** will operate a strategic control on the project, which includes, but is not limited to: (a) management/interactions/communications in/among WPs; (b) review and approval of all deliverables before their release outside the Consortium; (c) quality control of projects outputs; (d) dissemination and exploitation strategies of results; (e) promotion of gender equality. Whenever necessary, MB will propose and implement corrective measures concerning the work plan in the emergence of delays or deviations, according to the **contingency plan** reported in **Table 1**. Besides the described risks, other adverse and now unpredictable events could arise. In this case, the MB, once informed by the involved partner, will define a specific contingency plan and apply the possible best mitigation strategies. MB will be composed by P1, who chairs and convenes the board, and at least one member of each Partner. The **Advisory Board (AB)** will be a balanced group of stakeholders (note that some institutions and organisations already guaranteed this participation through supporting letters), which will help run the project better, keeping MB informed on various trends (e.g. legal issues) that may affect the project, but without voting rights on project matters. The **Dissemination Exploitation and Communication Board (DECB)** will manage the communication among partners and the activities related to the public diffusion of results, according to a specific DECP (see Exploitation and Dissemination section for a preliminary assessment). **General Assembly Meetings (GAM)** will be organised three times (at the beginning, middle and end of the project) possibly in conjunction with WATER4ALL meetings. For each WP and Task, WP and Task Leaders have been identified among the partners. WP Leaders will be responsible for the coordination of the different tasks within each WP, while Task Leaders will be the main driving force for the implementation of each task, translating decisions of the MB into daily management activities and reporting results and potential critical points to the WP Leader. WP and Task Leaders maintain contacts with all participants to the project activities at their proper level and ensure the flow of information within and among WPs.

Table 1 – Potential risk situations of WATERPATH and proposed mitigation measures (L=low; M=medium).

Type of potential risk	Description of risk	Risk Management - Contingency Plans
Consortium/Management risks	A Partner withdraws or is unable to provide a foreseen contribution (Probability: L, Impact: L)	A solid and thorough consortium agreement ensures that original commitments are maintained and such an unlikely situation is handled professionally. However, should such an issue occur, mitigating actions will be taken such as reassignment of tasks within the Consortium.
	Conflicts in the Consortium (Probability: L, Impact: L)	Conflicts are foreseen to be solved by a majority vote in the Project Management Board (P1 has the casting vote).
	High price increase due to inflation (Probability: M, Impact: M)	Re-planning of activities to offset price increases
	Unexpected delays in achieving deliverables (Probability: M, Impact: M)	WP and Task Leaders will monitor WP/Task progress to detect any delay at early stages
Technical risks	Complexity and variability of the ecosystem services studied (Probability: L, Impact: L)	Responsible partners will create a step-by-step process to identify potential problems and looking for corrective actions. Extra period of sampling will be identified.
	Low quality of measurements (Probability: L, Impact: L)	Review of the sampling and analysis protocols contained in the PMH and identification of the related corrective actions.
	Unexpected results, including ARB/ARG different time-scale responses (Probability: L, Impact: L)	In the presence of unexpected trends in risk assessment and meta-analysis, the consortium will evaluate the opportunity to re-scaling the collected data and modify the data analysis procedure in order to overcome this problem. Contextually, PMH and DMP will be updated.
	Insufficient quality of biochar for wastewater treatment (Probability: L, Impact: L)	Modification of the activation procedure, e.g. increase of the activation temperature and/or addition of activation reagents.

10. Time schedule and working programme (use a Gantt chart or equivalent).

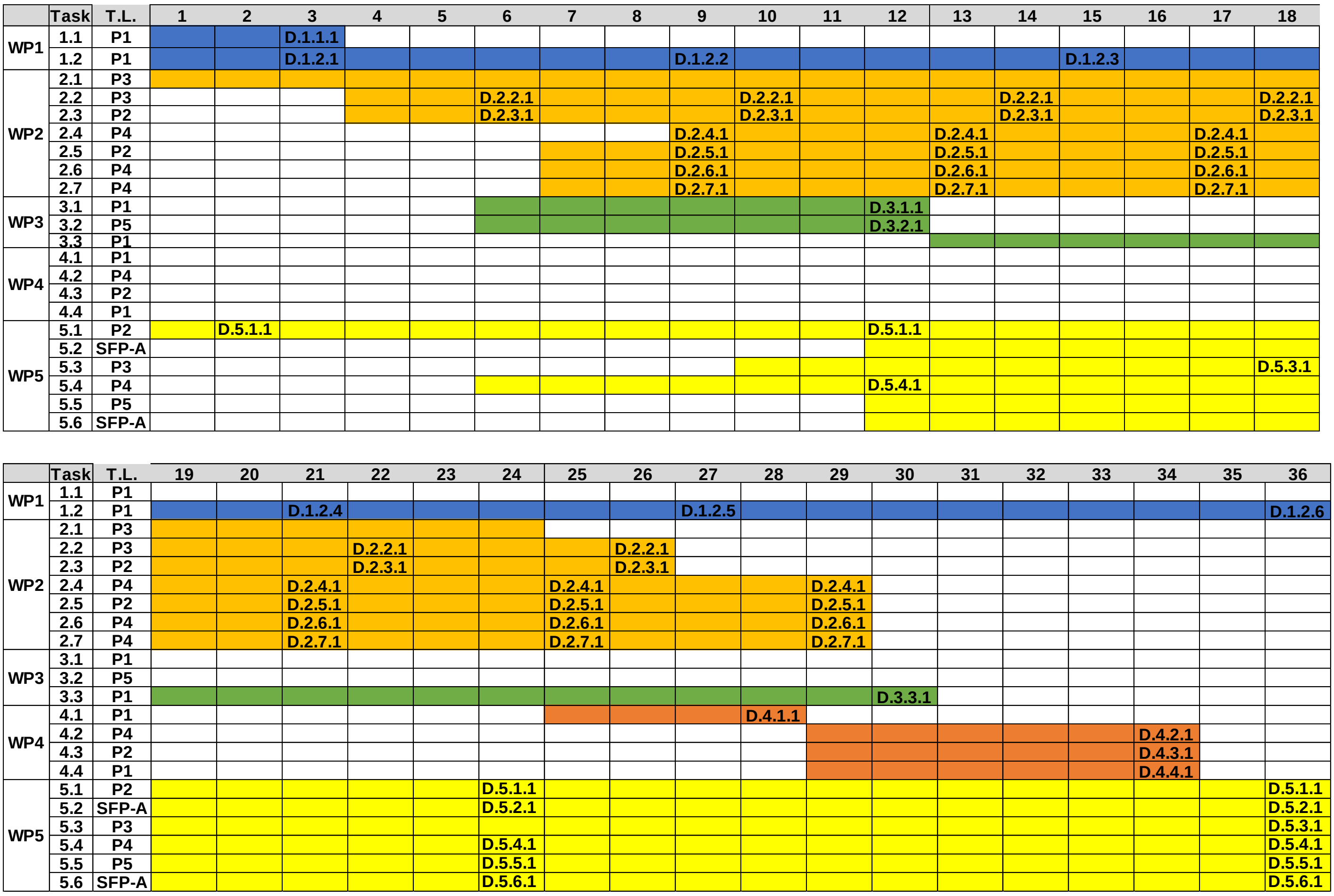

Figure 3 – The Gantt chart

11. Exploitation and dissemination of results. The exploitation and dissemination of results obtained by WATERPATH is based on the DECP reported in **Table 2**, with quantitative key performance indicators (KPIs).

Table 2 – WATERPATH DECP illustrating the dissemination, exploitation and communication (DEC) tools, target groups (mainly involved in bold), KPIs, and short/long-term impacts. Note that companies involved in the integrated cycle of water management are abbreviated as IWCCs.

DEC tools	***Target group(s)***	***KPI***	***Impacts***
Website	**Civil Society**; Policy makers; **Scientific Community**; **IWCCs**	500 visitors per year on average	Inform; Promote; Awareness; Engagement
Social Media (i.e., X; Instagram)	**Civil Society**; Policy makers; Scientific Community; **IWCCs**	> 2000 visits on average; >1000 followers on average	Inform; Promote; Engagement
Newsletters (1 per semester for a total of 6 issues)	**Civil Society**; **Policy makers**; Scientific Community; **IWCCs**	Distributed in e-form within the public area of the webpage and through the social media; > 1000 people	Knowledge transfer; Inform; Promote; Engagement

Public engagement (oriented to high-school students)	**Civil Society**	2 events in each country	Awareness; Inform
WATERPATH Database Publication	Civil Society; **Policy makers**; **Scientific Community**; **IWCCs**	2/3 access points (website and public repositories); > 1000 people	Knowledge transfer; Inform; Awareness
Communication campaigns (≥3 for the whole project period)	**Civil Society**; **Policy makers**; **Scientific Community**; **IWCCs**	≥ 3 Press releases; round tables, TV and radio (> 5000 people)	Inform; Promote; Engagement
Peer reviewed ISI-impacted scientific publications	Policy makers; **Scientific Community**; IWCCs	≥2 "Open Access" articles per year	Knowledge transfer; Inform;
Oral/poster communications in National/International scientific conferences	Policy makers; **Scientific Community**; IWCCs	≥3 contributions per year	Knowledge transfer; Inform;
Stakeholder oriented demonstration sessions (including videos on Social Media)	Policy makers; **Scientific Community**; **IWCCs**	50-100 participants working on same or related topics	Promote; Awareness; Engagement;
"After WATERPATH" meetings	**Civil Society**; **Policy makers**; **Scientific Community**; **IWCCs**	1 per country (with > 5 policy makers and IWCCs per meeting)	Knowledge transfer; Inform; Promote; Engagement.

11.A – Stakeholders' engagement. WATERPATH carried out an in-depth *a priori* identification of organizations representing relevant stakeholders in each country of the consortium, linking the specific objectives of the project to the specific interests of the stakeholders, which are policy-makers or public/private companies responsible for the integrated cycle of water management. As mentioned in Section 2 these institutions and companies have already agreed to be involved in the project by signing letters of support. **Table 3** summarizes the stakeholders already involved, their typology and the rationale for their involvement.

11.B – Open science practices, sharing and management of research outputs and Data Management approach. WATERPATH pursues an **"Open Science" approach** to research, based on open cooperative work that emphasizes the sharing of knowledge, results and tools as early and widely as possible. According to European guidelines, WATERPATH will operate on the principle of being "as open as possible, as closed as necessary". This means that results and data will be kept closed if making them public in open access is against the researcher's legitimate interests, as detailed in the Consortium Agreement (see also Section 4 for IPR). However, WATERPATH will adopt the "Open Access" practice of providing online access to scientific information that is free of charge and reusable to the user. This will include peer-reviewed publications, but also WATERPATH data and datasets, following the FAIR (Findable, Accessible, Interoperable and Re-usable) principle. A **Data Management Plan (DMP)** will be prepared according to the FAIR approach as part of PMH (see Task 1.2), detailing what data the project will generate and how it will be exploited and stored. A simple collaboration platform for storing and sharing data, information, exchanging messages and working on the deliverables will be set up. A first version of the DMP will be delivered within the first 3 months of the project. Since the DMP is expected to mature during the project, more versions will be included at later stages.

11.C – Engagement of citizens, civil society and end users where appropriate. Although WATERPATH is an advanced research project, the topics covered can be the subject of appropriate communication activities towards citizens, civil society, and end users of the ecosystem services studied. In fact, all citizens are the end users of water ecosystem services investigated by WATERPATH, since they use continuously potable water as food or for other daily activities, eat agricultural products grown through irrigation with freshwater or, in some cases, with TWWs, and use aquatic ecosystems for recreational activities. The DECP (**Table 2**) includes a series of dissemination activities that aim to reach civil society.

Table 3 – Stakeholders engaged for WATERPATH and rational for their involvement. Note that companies involved in the integrated cycle of water management are abbreviated as IWCCs.

Stakeholder	Category	Reasons to involve the Stakeholders	Why the stakeholder are willing to be involved
Tuscany Region – Directorate for the Environment, Circular Economy, Soil Protection, Public Works, and Civil Protection, Florence, Italy. Reference person: Dr. Monia Monni, Councilor.	Government policy maker.	Support in the identification of the most correct environmental policies and ensure the relevance of research outputs. Contribution to the project through participation in the WATERPATH Advisory Board.	Opportunity to develop policies based upon rigorous scientific knowledge. Update of the regional "Water Protection Plan" in response to the requirements of the Water Framework Directive. Contribution to the European policy (e.g., monitoring watch lists).
Piedmont Region – Directorate for the Environment, Energy and Land Use, Turin, Italy. Reference person: Dr. Stefania Crotta; Director.			
Consejo Insular de Aguas de Gran Canaria, Las Palmas de Gran Canaria, Spain. Reference person: Dr. Carmelo Javier Santana Delgado, Director.			
Mureș Water Basin Administration, Târgu Mureș, Romania. Reference person: Eng. Sorin Vlad, Director.			
Publiacqua S.p.A, Florence, Italy. Reference person: Dr. Daniela Santianni, Director of the laboratory for analysis of drinking water and wastewater.	IWCC	Sharing of technical expertise in the sectors of (i) potabilization, (ii) wastewater treatment, and (iii) drinking water and wastewater analysis. Assistance in sampling environmental waters and wastewaters. Contribution to the project through participation in the WATERPATH Advisory Board.	Possibility of networking with potential new customers through the engagement process. Possibility of improving the efficiency and profitability of potabilization and wastewater purification processes.
Empresa Mixta de Aguas de Las Palmas S.A., Las Palmas de Gran Canaria, Spain. Reference person: Dr. Mercedes Fernández Couto Gómez, General Director.			
Compania Aquaserv S.A., Târgu Mureș, Romania. Reference person: Eng. Sipos Levente, General Director.			

Among these, of particular importance are (i) the website which will host a public area intended for dissemination towards the social community and (ii) some civil society-oriented communication campaigns which will have the aim of informing the social community of the WATERPATH results. It is also worth noting the planning of public engagement events oriented to high-school students, which will have not only the aim of informing this important part of civil society, but also of stimulating their interest in environmental issues with a view to their future involvement as university students and subsequently as workers in environmental sciences.

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