# Dissemination report Year 3 (D7.2.3)

Factsheet and Best Management Practices
NPK optical sensor and Biosensor PPP

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## List of abbreviations

| | |
|---|---|
| AGRINUPES | Integrated monitoring and control of water, nutrients and plant protection products towards a sustainable agricultural sector |
| BMP | Best Management Practices |
| CRU | Cascade Re-Use System |
| DPV | Differential Pulse Voltammetry |
| DSS | Decision Support System |
| DUVA | Direct Ultra-Violet Assessment |
| EC | Electrical Conductivity |
| ET | Evapotranspiration |
| ELV | Emission Limit Values |
| GAP | Good Agricultural Practices |
| GEM | Greenhouse Emission Model |
| GUI | Graphical User Interface |
| ISE | Ion-Selective Electrodes |
| K | Potassium |
| $K^+$ | Potassium ion |
| LOD | Limit of Detection |
| LOQ | Limit of Quantification |
| MIS | Micro-Irrigation Systems |
| N | Nitrogen |
| $NO_3^-$ | Nitrate |
| $NO_2^-$ | Nitrite |
| NPK | Nitrogen, Phosphorous, Potassium |
| P | Phosphorous |
| PLS | Partial Least Squares |
| $PO_4^{3-}$ | Phosphate ion |
| PPP | Plant Protection Product(s) |
| RSD | Relative Standard Deviation |
| SME | Small and Medium Enterprises |
| SPGE | Screen-Printed Gold Electrodes |
| WFD | Water Framework Directive |
| ZLD | Zero Liquid Discharge |

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# 1. Summary

This report gives a general introduction on innovative optical based sensors for measuring nutrients (NPK) and biosensors for plant protection product monitoring (imidacloprid and pirimicarb). Preliminary data of the performance of these sensors is presented in two factsheets. For end-users of the sensors, like growers, advisory services and water authorities, also best management practises for using the sensors in a number of use cases are described.

The sensors offer possibilities for horticulture growers to monitor the quality of their input and output water flows, for semi-open cropping systems as well as for those having a closed water system, with recirculation of the surplus nutrient solution (*e.g.*, drainage). It will help them to optimize their use of water, fertiliser and plant protection products to minimize the environmental impact.

This report gives a short introduction on the sensors and their use. For more detailed descriptions of the technologies and use cases, the reader, *e.g.* technology providers, should refer to other deliverables or publications from the project. It describes the factsheets and best management practices for the use of an NPK optical sensor and a PPP Biosensor and its related products. The information therein is the final result of the knowledge obtained from the AGRINUPES project.

The new sensors may lead to new worldwide markets for the European water technology sector, thus strengthening the competitiveness and growth of SME and related companies. As a result, significant increase of water and fertilizer use efficiency may be obtained in the agricultural/horticultural sector, longer and economic reuse cycle for the drainage water may be achieved, and pollution of water bodies and soil degradation by fertilizers and plant protection products can be prevented or significantly reduced.

# 2. Introduction

For optimizing crops' needs while minimizing the environmental impacts, sustainability and competitiveness of European agriculture are intrinsically related to the efficient use of water, fertilizers and Plant Protection Products (PPP). Good Agricultural Practices (GAP) - in the context of the circular economy - forces growers to minimize their wastewater and thus optimize the use of NPK-based fertilizers and apply a safer use and handling of PPP. Better management requires reliable Decision Support Systems (DSS) based on water quality feedback making use of cost-effective, robust, low-maintenance, easy to use and accurate sensors for nutrients and PPP. So far, available sensor technology does not meet the challenges for on-site monitoring.

AGRINUPES has been developing ion selective nutrient (NPK) sensors for use as on-line feedback system for water and nutrient management systems in horticulture and demonstrated their use for practical management purposes at several European demonstration sites. The innovative optical based NPK-sensors can be used by growers in horticulture targeting optimal water and nutrient supply and reuse, minimizing the effects on the environment and allowing to reduce pressure on the utilization of water resources. Suppliers of technologies and systems for water and nutrient management (fertigation controllers, water cleaning systems) can implement the sensor additional to their existing systems as sensor for feedback control application, thus adding value to their products.

In addition, AGRINUPES has been developing a biosensor for detection of PPP residues in waters, namely imidacloprid and pirimicarb, which are commonly used in plant protection. Growers may use these sensors to check the recirculated water, wastewater and if available the performance of their water cleaning equipment and possible malfunctioning to avoid hazards like unwanted emissions. Governmental organizations like water authorities may use the sensors for checking water quality (PPP residues and nutrient content) in ground and surface waters and compliance to norms regarding maximum levels of nitrogen and phosphorus in wastewaters.

Both sensor types were tested, validated and demonstrated in five case studies throughout Europe. Technology suppliers (manufacturers and re-sellers of equipment for agricultural practices) may use the AGRINUPES outcome for developing their activity. The new sensors may lead to new worldwide markets for the European water technology sector, thus strengthening the competitiveness and growth of SME and related companies. As a result, significant increase of water and fertilizer use efficiency may be obtained in the agricultural/horticultural sector, longer and economic reuse cycle for the drainage water may be achieved, and pollution of surface and ground waters by fertilizers and PPP can be prevented or significantly reduced.

This dissemination report gives a general introduction on both sensor types. Preliminary data of the performance of these sensors is presented in two factsheets. For end-users of the sensors, like growers, advisory services and water authorities, also BMP are described as were developed in the five case studies. The report reflects the progress in the project until the end of the project (M45) after finalising a number of (semi-)practical tests and demonstrations.

# 3. Optical sensor for nutrients (NPK)

## State of the art and innovation

Crop water and nutrient requirements are decisive parameters to be considered in fertigation scheduling. Research efforts have been made to identify how to optimally correct the nutritive solution. A great challenge is the nutrients replenishment as plants grow, without affecting their concentration balance in the nutritive solution. It is expected that these data may be acquired at a suitable sampling frequency for continuous monitoring and control, instead of usual lab-based analysis.

Most state-of-the-art systems for automatic fertigation control are based on pH and Electrical Conductivity (EC). However, EC is an indiscriminate measure for the total nutrient composition. Several of the larger fertilizer mixers such as Netajet series (NETAFIM™12) and the FertiMix (HortiMax, NL) use a pH-EC fuzzy logic control [1] in their customizable controller [2]. This topic has motivated the development of electrochemical sensors, such as Ion-Selective Electrode (ISE), with deep investigation in past decade [3]. These works concluded with the motivation for research on stability, maintenance and robustness improvements of the measurement system (fault tolerant) and a better life expectation for the sensors.

Plants need macronutrients and micronutrients, but it is difficult to measure all of them. Thus, a regular check on the availability of at least the major nutrients (NPK) is recommended to effectively control the application. Although some electrochemical-based multi-ion probes are commercially available [4], recent works [5] demonstrated that these sensors have several drawbacks (*e.g.*, need frequent calibration, etc.) and therefore they are not suitable for feedback control. In this context, optical methods have long been established as suitable analytical techniques for many complex species. Especially for nitrates, several *in-situ* systems have been established based on direct UV spectrophotometric evaluation or colorimetric methods. Although these systems have good reliability, the method is not yet used as most reported systems still rely on other complex techniques, mostly based in wet chemistry and multistep operation.

Latest developments associating microfluidics with low cost optoelectronics and the versatility of optical fibres, promise to deliver a low cost, compact system suitable for online monitoring in the field [6]. Indeed, a wide diversity of fibre based chemical and biosensors has been developed for diverse applications [7]. Optical fibre-based sensors development for NPK ions was the objective of a project with funding from the National Institute of Food and Agriculture [8]. Moreover, there are solutions [9] for $Ca^{+2}$ and $Na^{+}$, but they are not commercially available, which motivates development for other easy-to-use, feasible and novel sensors technology.

In this context, AGRINUPES explores novel approaches in the design of low-cost optoelectronic platforms, developed in recent projects [10,11], suitable for field deployment.

## Sensor description

A compact all-in-one monitoring system was developed for real time determination of nitrogen, phosphorus and potassium (NPK) in nutrients fertilizer water [17]. Direct UV-Vis spectroscopy combined with optical fibres was employed to record absorption spectra of nutrient solutions resulting from local producer samples. The schematic principle is shown in Figure 1.

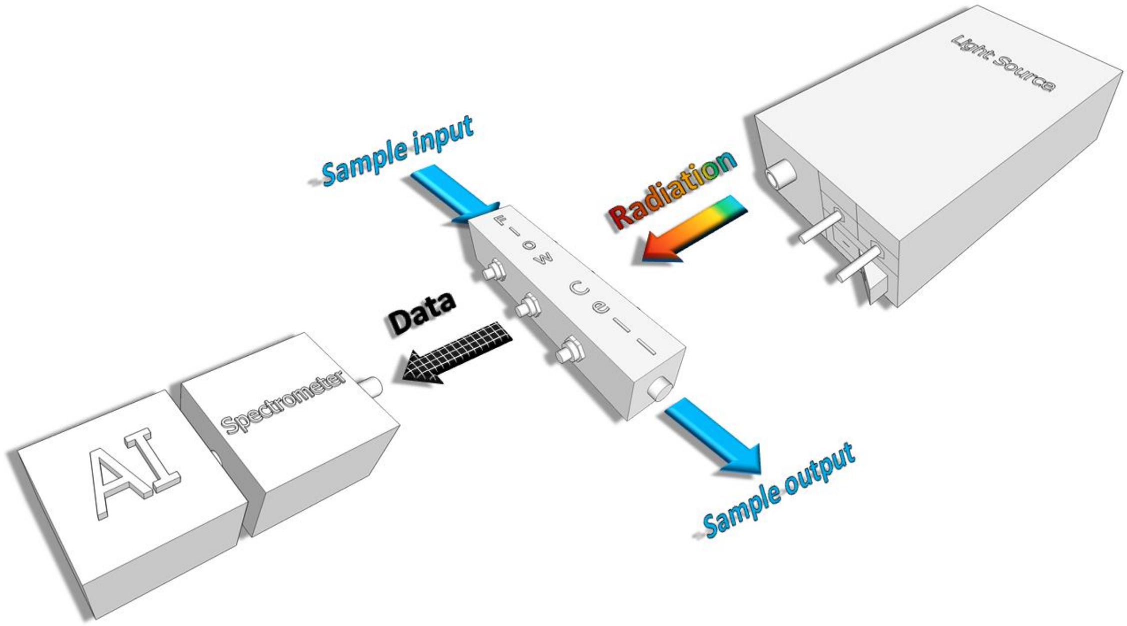


***Figure 1. Schematic composition of a compact, portable and low-cost prototype system.***

A compact benchtop system was built using a FiberLight® high-power light source, a custom-built sampling chamber with an automatic pumping system and an Ocean Insight® STS-UV spectrometer as a detector. Transmission optical fibres were used for signal acquisition and transmission, as shown in


***Figure 2. Prototype optical NPK-sensor unit.***

## Read-out unit

The high-level architecture of the sensing systems is shown in Figure 3. The system interfaces with a flow system carrying the irrigation waters by means of a probe system, which is excited and interrogated by means of adequate optoelectronic modules most of the times using an optical fibre of adequate length and characteristics adapted to the sensor optical properties. The whole system is controlled by electronic modules where control, signal processing and calibration function are applied, allowing to obtain the concentration values of the relevant species.

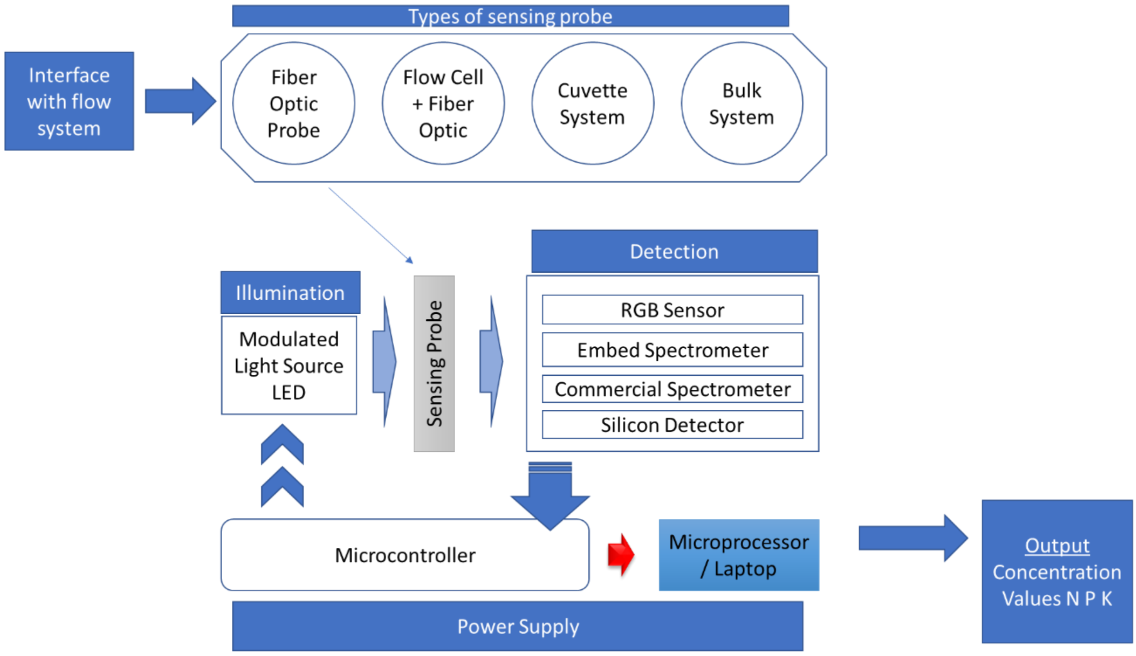


***Figure 3. Diagram showing the high-level architecture of the modular sensing system.***

Upon irradiation of the sample with ultra-violet to visible wavelengths, it is possible to extract information of the optical properties of the sample itself. The data is transmitted by the optical fibres to the spectrometer module, where the data acquisition is performed by custom-designed software. The versatile programming environment used (LABView from National Instruments) also enables the same custom software to control component parameters on a friendly graphical user interface (GUI). (see snapshot of user interface in Figure 4).


*Figure 4. Example of the current welcome screen of the custom developed GUI.*

## Test results

The prototype was preliminary tested for nitrate under laboratory conditions (D3.3) using artificially made samples. Results are shown in Figure 5. The result shows a promising linear behaviour, considering the optimum spectroscopic absorption value for $NO_3^-$.

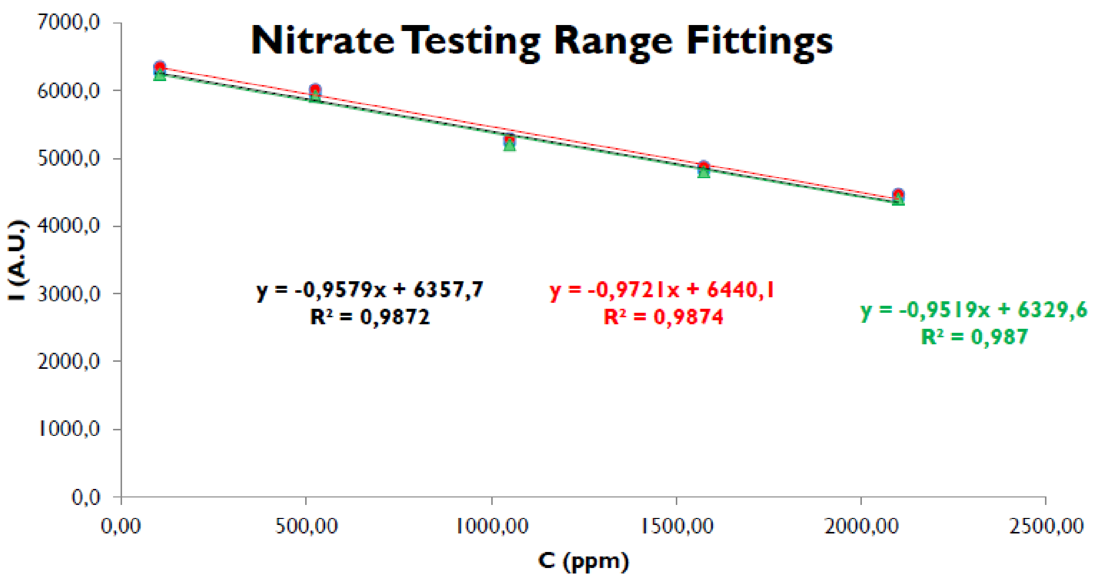


***Figure 5. Function test for Hoagland solutions with different concentrations for nitrate.***
*(Sweden, June 2019)*

To make an actual calibration curve, absorbances were obtained using 4 $NaNO_3$ solution concentrations (100, 250, 500 and 2000 ppm). Results are shown in Figure 6.

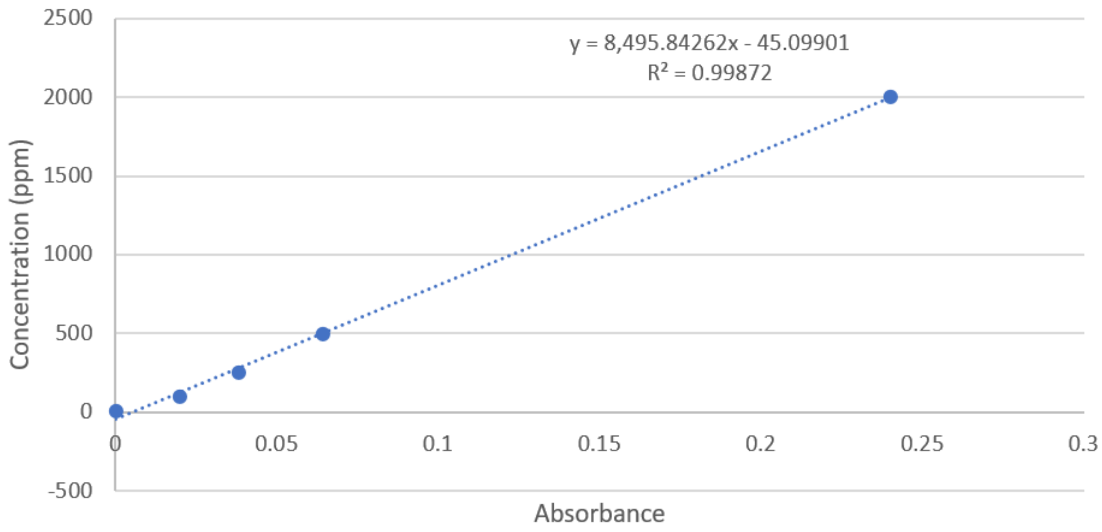


***Figure 6. Nitrate calibration curve (RISE lab Växjö, Sweden, 05-02-2020).***

To evaluate the nitrate monitoring calibration under practical conditions, water samples originating from the greenhouse facilities at Bleiswijk (The Netherlands) were used. As reference, the composition of the water samples was also determined by an analytical lab. Based on measured absorbances and the calibration curve, results were obtained as shown in following tables.

***Table 1. Nitrate concentration of practical greenhouse samples (WUR, Bleiswijk, NL) as measured with the calibrated NPK-prototype sensor.***

| Sample | Absorbance | Calculated concentration (ppm) | Lab measurement (ppm) | Lab measurement (mmol/l) |
|---|---|---|---|---|
| 301 drain | 0.32 | 2674 | 211 | 3.4 |
| 301 gift | 0.31 | 2589 | 372 | 6 |
| 907 blok A | 1.05 | 8876 | 372 | 6 |
| Geofood 5.04 | 0.75 | 6327 | 1048 | 16.9 |
| 303 drain | 0.49 | 4118 | 1823 | 29.4 |

***Table 2. Composition of the water samples.***

| | pH | EC | NH4 | K | Na | Ca | Mg | Si | NO3 | Cl | SO4 | HCO3 | P | Fe | Mn | Zn | B | Cu | Mo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Units | | | mmol/l | mmol/l | mmol/l | mmol/l | mmol/l | mmol/l | mmol/l | mmol/l | mmol/l | mmol/l | mmol/l | µmol/l | µmol/l | µmol/l | µmol/l | µmol/l | µmol/l |
| 301 gift | 7.3 | 1.5 | < 0.1 | 7.1 | 0.5 | 2.6 | 1.1 | < 0.1 | 6 | 0.5 | 2.3 | 3 | 0.22 | 68.6 | 14.1 | 9.2 | 8 | 1.2 | 0.49 |
| 303 drain | 5.9 | 8 | 0.9 | 28.1 | 1.5 | 20 | 13.2 | 0.5 | 29.4 | 15 | 24.3 | 0.6 | 2.5 | 30.5 | 1.8 | 4.9 | 92 | 1.1 | 0.64 |
| 907 BlokA | 7.3 | 1.5 | < 0.1 | 7.1 | 0.5 | 2.6 | 1.1 | < 0.1 | 6 | 0.5 | 2.3 | 3 | 0.22 | 68.6 | 14.1 | 9.2 | 8 | 1.2 | 0.49 |
| geofood 504 | 6 | 2.6 | < 0.1 | 11.2 | 1.5 | 3.7 | 1.5 | < 0.1 | 16.9 | 2 | 1 | 0.4 | 1.3 | 64.2 | 5.1 | 4 | 37 | 0.7 | 0.49 |
| 301drain | 7.4 | 4.6 | < 0.1 | 13.3 | 1.3 | 12 | 9.9 | 0.3 | 3.4 | 7.8 | 22.8 | 3.1 | 0.62 | 34.6 | 1.7 | 11.3 | 77 | 1.1 | 1.2 |

As seen, the calculated concentrations are much higher than the actual concentrations which were measured by the lab. Furthermore, there is no clear correlation between the measured absorbance and the real concentrations. As such, it was concluded that the linear calibration does not seem to work properly for determining the nitrate concentration in a 'real' greenhouse water sample. This was to be expected as several factors exist that may condition the adequate estimation of concentration values. Since greenhouse samples have several contributions from different components, higher complexity calibration solutions should be tested in order to assess this methodology, as using single species solutions over-simplifies the whole sample complexity.

Feasibility of the system, also for Potassium and Phosphorus, was also studied and reported in [17] by INESTEC. N, P and K spectral interference was studied by mixtures of commercial fertilizer solutions to simulate real conditions in hydroponic productions. This study also demonstrated that the use of bands for the quantification of nitrogen with linear or logarithmic regression models does not produce analytical grade calibrations. Furthermore, multivariate regression models, *e.g.*, Partial Least Squares (PLS), which consider specimens interference, perform poorly for low absorbance nutrients. The high interference present in the spectra has proven to be solved by an innovative self-learning artificial intelligence algorithm that is able to find interference modes among a spectral database to produce consistent predictions. By correctly modelling the existing interferences, analytical grade quantification of N, P and K has proven feasible. The results of this work open the possibility of real-time NPK monitoring in Micro-Irrigation Systems.

## Factsheet: NPK-sensor

This factsheet gives data of the performance of the AgriNuPes NPK-sensor based on the outcome of laboratory and semi-practical testing. Data should be considered as preliminary, as extensive testing under real-practical conditions have not been performed.

***Table 3. General description of the optical NPK-sensor (targeted[1])***

| | Description |
|---|---|
| What | Optical sensor for on-line sensing of Nitrogen, Phosphorus and Potassium including a light source and a spectrometer connected with optical fibres. |
| Features | • Large dynamic range suitable for horticulture applications<br>• High stability and low drift<br>• Long lifetime<br>• High accuracy and reliability<br>• Low temperature and pH sensitivity (yet to be tested)<br>• No need for re-calibration (low maintenance)<br>• Compact<br>• Low cost<br>• Multi-analyte calibration option (not yet available) |
| Boundary conditions | *To be defined.* |
| Target Audience | Technology suppliers, Farmers, Greenhouse growers, Nurseries, Technicians, Advisory Services, Water Authorities, Scientist/Researchers. |
| Accessibility | Institute for Systems and Computer Engineering, Technology and Science, Porto, Portugal (INESC TEC). |
| Disclaimer | This factsheet contains preliminary information and no rights can be deduced from the given specifications. |

[1] Targeted features are not yet achieved in tested prototypes. Most test still need to be done (status M45).

*Table 4. Detailed specifications of the optical NPK-sensor*
*(for sensors when used with the accompanying optical read-out platform)*

| Specification | Target[2] (Reference) | Actual (tested)[3] |
|---|---|---|
| **Measuring ranges** | | |
| N ($NO_3^-$, $NO_2^-$) | 5-30, (0-100, 0-10) mmol/l[4] | *$N_{Total}$= 1.66 – 8.95 mmol/L* |
| P ($PO_4^-$) | 0-5 (0-50) mmol/l | *$P_{Total}$= 0.16 – 5.31 mmol/L* |
| K ($K^+$) | 0-10 (0-50) mmol/l | *K= 2.91 – 13.21 mmol/L* |
| **Operating conditions[5]** | Nearly no interference | *Not available* |
| Ballast ions (Na+, $Ca_2^+$, $Cl^-$) | (0-150, ---, 0-100) g/l | |
| ($BO_3^-$) | (0-2) g/l | |
| Organic compounds (roots, exudates) | No influence of organic matter | |
| Chemical compounds (PPP) | No other influences of chemical composition | |
| Operating temperature | 10-40°C | |
| **Performance** | | |
| Accuracy | Per mmol/l | Per mmol/l |
| Response time | < 15s | < 15s |
| Drift | < 0.5 mmol/l | - |
| Cross-sensitivity | K vs. Na | untested |
| Repeatability | < 0.5 mmol/l difference | - |
| Lifetime | > 6 -12 months | > 12 months |
| Temperature influence | < 1 °C | - |
| pH influence | 4.5-6.5 (2-10) | 2.39 – 8.79 (tested) |
| Selectivity | - | - |
| **Construction** | | |
| Materials: | Low pH = 3, no metals | Low pH = 3, no metals |
| Resistant to: | - | - |
| Dimensions: | 15cm long, 1 cm diameter | 36×16×11 cm |
| Cable length (cm): | 50-100 cm | user customizable |
| **Optical Readout Platform** | | |
| Materials: | No metals | 12V-powered peristaltic pump |
| Dimensions: | - | 5V USB interface system for control |
| Power (V, mA): | - | |
| Connection: | - | USB, serial to PC |
| Operating temperature: | - | +5 – 50 °C (spectrometer) |

[2] The specification above was compiled for measuring in water in the soilless situation (NL-case). Ranges may vary strongly depending on the type crop production system (open, closed), the crop type, as well as the location where the sensors are installed in the irrigation system. This list must still be elaborated for all other case studies.
[3] This column will give the final specification as currently achieved with test set-ups. In the Year 3 version (Final) of this document it will give the final specifications.
[4] Horticulture hydroponics measures in mmol/l; others measure in mg/l (N, $P_2O_5$, $K_2O$.).
[5] A full description of how to measure in a water sample and how to prepare the water sample must be added.

# 3. Biosensor for Plant Protection Products

## State of the art and innovation

Regarding sensing systems for plant protection products (PPP), there is an urgent need for a quick and cheap way of detecting PPP in waters. No sensors are available for detection of PPP residuals in water today, so advanced chemical analyses by certified labs are required. These analyses are both time consuming and expensive, preventing extensive surveillance of surface waters. In this context, AGRINUPES explores novel approaches in the design of PPP-sensors using the electrochemical biosensor technology. The aim is to commercialise this type of biosensor for direct detection of imidacloprid and pirimicarb in a detection kit using disposable electrode. This kit should be usable for on-site monitoring of water pollutants without training end users, be inexpensive, practical, portable and have a lifetime longer than 1 year. They require a high accuracy, a low temperature and pH sensitivity, and need no re-calibration. The sensors will be used in practise by farmers, greenhouse growers, nurseries technicians, advisory services, water authorities, and scientists/researchers. Technology suppliers will take up the prototypes and bring it to the market.

## Sensor description

Due to their low cost, simple fabrication, small size, and portability, biosensors are excellent candidates for the design of detection systems for PPP [19, 20]. Point of care (POC) tests are tests using for detection of analytes as nucleic acid, proteins, dissolved ions and gases, drugs in samples as serum, urine, saliva [33]. These tests are widely used around the world due to have much excellent properties. Recently, biosensors have used in POC analysis due to being specific, portable and low cost [21]. Biosensors are remarkable instrument in different areas [22] as clinical, environmental and food analyses. Working principles is based on biochemical reaction between analyte and biomolecules [21].

AGRINUPES uses aptamers instead of antibodies. Aptamers are chemical antibodies and single stranded DNA molecules that can bind targets with high selectivity and specificity. Nucleic acid aptamers that can be chemically synthesized are superior to antibodies in terms of stability at ambient temperature, cost and ease of chemical modification. Aptamers do not require the use of biological systems for their production, which minimizes batch-to-batch variation. Consequently, they are better tools for the construction of sensors by making them more efficient, easy-to-obtain/produce as well as more reliable.

Aptamers with high affinity to imidacloprid and pirimicarb have been selected and prototype sensors were designed and tested using artificially made water mixtures in the laboratory by using an electrochemical-based test system. Now, the prototypes are ready for testing with real process water samples from the agricultural domain.

## Aptamer selection

Aptamers were produced for the selective recognition of imidacloprid and pirimicarb. As shown schematically in Figure 7, the thiol-modified aptamer was immobilized onto a gold electrode and then treated with the sample. The affinity of a thiol group towards a gold electrode forms the basis of immobilization process.

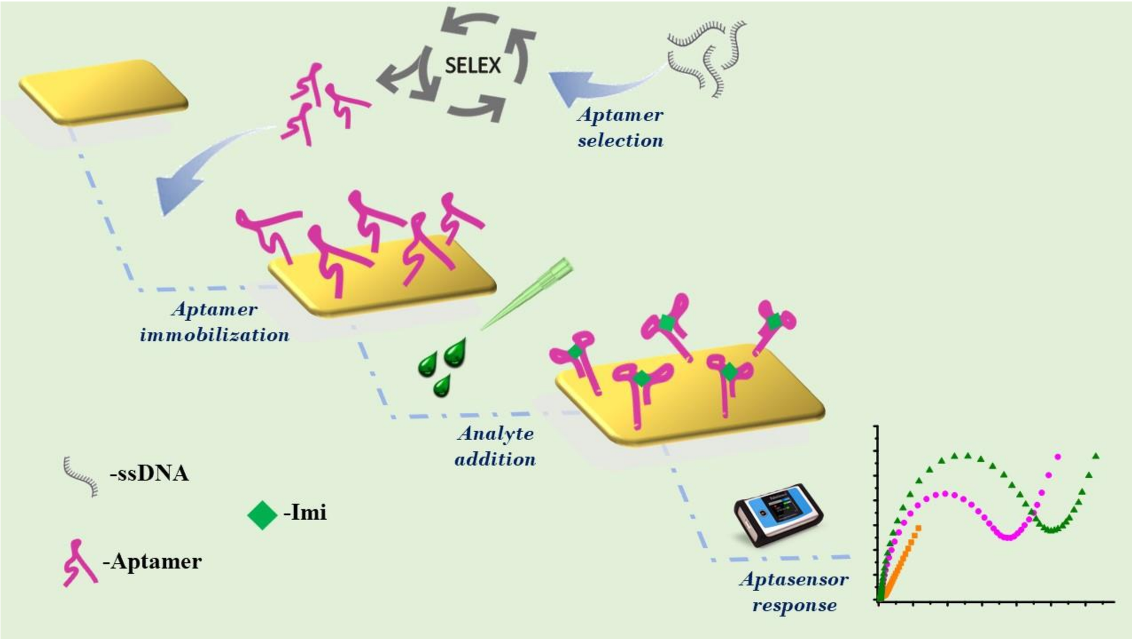


***Figure 7. Representation of the surface preparation and the basis of measurement.***

## Test results

For detailed characterization of the recognition features of the aptamers, electrochemical sensors prototypes were produced (Figure 8). Functional testing of these prototypes and obtaining a calibration curve for imidacloprid is described in [18]. Differential pulse voltammetry (DPV) was carried out using a PalmSens Potentiostat (Palm Instruments, The Netherlands).

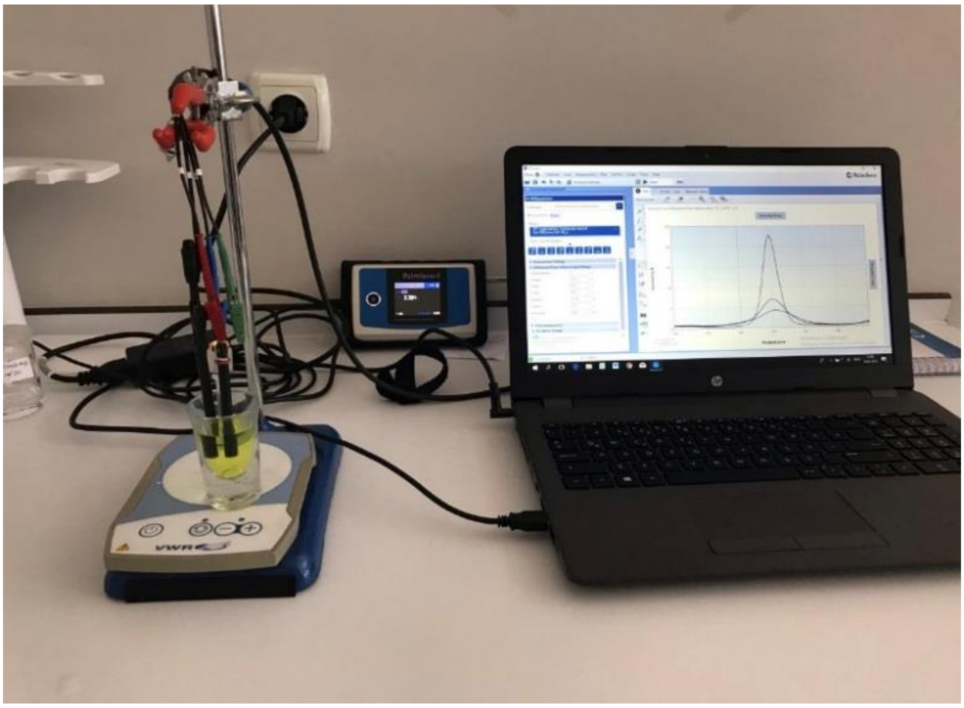

***Figure 8. The measurement set-up for testing the biosensor.***

DPV is an electrochemical technique where the cell current is measured as a function of time and as a function of the potential between the indicator and reference electrodes. The potential is varied using pulses of increasing amplitude and the current is sampled before and after each voltage pulse. The difference between current measurements at these points for each pulse is determined and plotted against the base potential.

A 3-electrode system was used to evaluate the synthesized aptamer performance. The system consists of an Ag/AgCl as reference electrode, platinum electrode as a counter electrode and a gold electrode as working electrode (Figure 8). Experiments were carried out in an electrochemical cell holding $Fe(CN)_6^{3-/4-}$; 5.0 mM, in 0.1 M KCl. In order to investigate the analytical performance of Au electrode/aptamer/pesticide electrode, the DPV technique was applied to the electrodes with varying concentrations of Imidacloprid.

The gold electrodes were treated with a chemical immersion in $H_2SO_4$, 10 cycles of cyclic voltammetry (CV) were carried out between -1.5 and + 1.5 V at 50 mV/s. Cycling the electrode potential in sulfuric acid solution is one of the most common electrochemical cleaning techniques. After chemical treatment, the gold electrodes were polished with 1.0, 0.5 and 0.3 µm alumina slurry followed by rinsing with distilled water and sonication in pure ethanol/water (1:1) for 2 min. Finally, the electrode was rinsed thoroughly with distilled water. Polished gold electrode surface was coated with 10 µL of aptamer (25 µM), prepared from 100 µM stock solution, by adequate dilution with phosphate buffer containing 5.0 mM $MgCl_2$ (10 mM, pH 7.4) and allowed to dry for 1 h at room temperature. This step allowed the creation of Au-SH linkage between the electrode and the aptamer. After, the electrode was thoroughly rinsed with water to remove any unbound aptamer. Finally, the electrochemical response signal of aptasensor was tested by dropped analyte (10 µL) with known concentrations on the surface and allowed to incubate for 30 min.

Afterwards, DPV measurements were conducted to evaluate analytical performance of the aptasensors. Throughout the study, all the data related to analytical performance were obtained from DPV measurements by using a water soluble redox probe ($Fe(CN)_6^{3-/4-}$ in 5.0 mM, in 0.1 M KCl) with a potential range of -0.4 to +0.8 V. Differential pulse voltammetry signals were recorded before and after the treatment of the surface with the analyte.

### *Imidachloprid*

Various concentrations of Imidacloprid (0-100 mg/nL) were applied to evaluate analytical performance of the sensor. The calibration curve is shown in Figure 9. Linearity was obtained between 0.1-50 ng/mL and linear equation of curve was determined as y=0.018x+0.333 ($R^2$=0.988). The signal decrease after 50 ng/mL concentration indicated that the sensor surface reached the saturation point with an increase in the amount of analyte. In addition, standard error of slope value and intercept value were calculated as 0.001 and 0.029, respectively. The limit of detection (LOD) and repeatability were also determined to examine analytic performance of the sensor. The repeatability was calculated with 9 successive measurements. The standard deviation (SD) and coefficient of variation were calculated as 0.056 and 3.65%, respectively. Furthermore, the LOD was found to be 0.19 ng/mL. Moreover, reproducibility of electrode-to-electrode was also investigated and a relative standard deviation (RSD) value was determined as 4.46% from measurements made for 3 different sensors in various days.

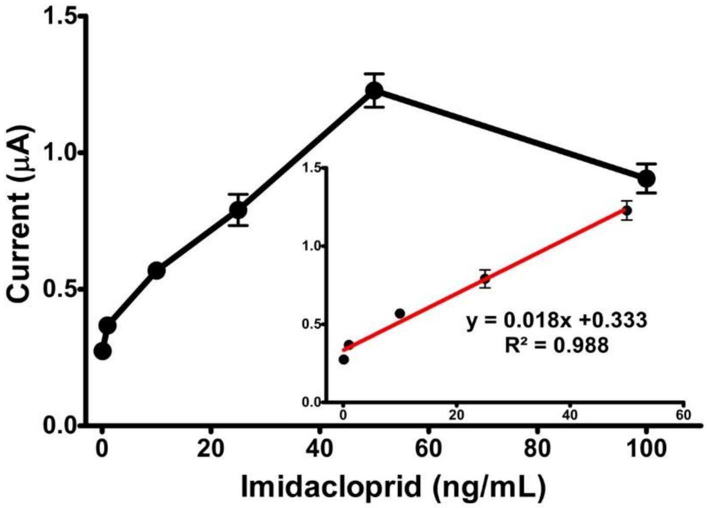


***Figure 9. Calibration curve of gold electrode/Imi-21 Aptamer for Imidacloprid.***
*Generated by DPV technique, error bars shows ±SD.*

For determination of selectivity of the sensor, additional experiments were conducted with possible interfering molecules such as an Imi and Carb mixture, 6-CN, Carb, wastewater, thiamethoxam and thiacloprid. Under the same experimental conditions, 10 ng/mL of each possible interferent molecule was added to the aptasensor surface. Responses were found as 104.6% for Imi-Carb mixture; 64.45% for 6-CN; 9.36% for Carb; 9.16% for wastewater; 9.33% for thiamethoxam and 14.81% for thiacloprid (Figure 10).

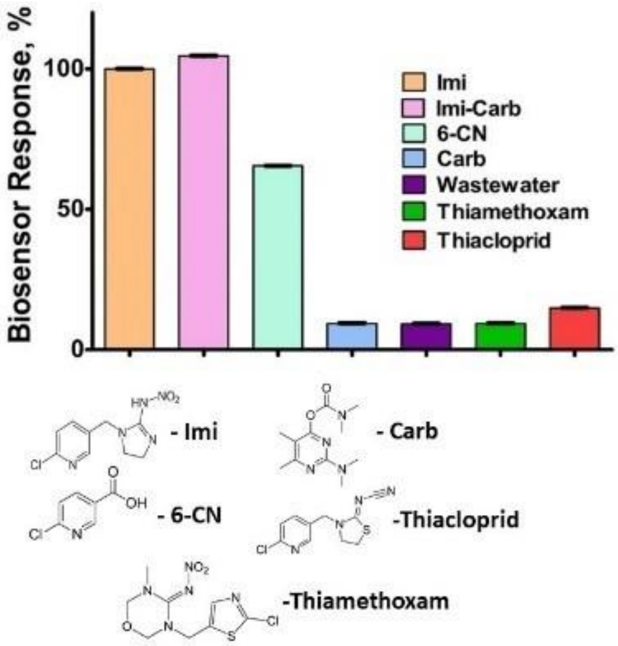


***Figure 10. Response of the Imidacloprid sensor for different interference molecules.***

The designed aptasensor platform showed lower response to the selected interference molecules. However, the response to 6-CN was the highest among the others. Since aptamers fold into unique structures and bind their targets via non-covalent interactions, we hypothesized that the common aromatic ring of Imi and 6-CN could be important for aptamer recognition. The aptasensor response to thiacloprid possessing the same aromatic ring was much lower, but interestingly it was slightly higher than the responses to the structurally different Carb and thiamethoxam.

### *Pirimicarb*

Various concentrations of Pirimicarb (0.1-10 ng/mL) were applied to evaluate analytical performance of the sensor. The calibration curve is shown in Figure 11. Linearity was obtained between 0.1-5.0 ng/mL and linear equation of curve was determined as y=0.485x+4.683 ($R^2$=0.984). The signal decrease after 5.0 ng/mL concentration indicated that the sensor surface reached the saturation point with an increase in the amount of analyte. In addition, standard error of slope value and intercept value were calculated as 0.044 and 0.125, respectively.

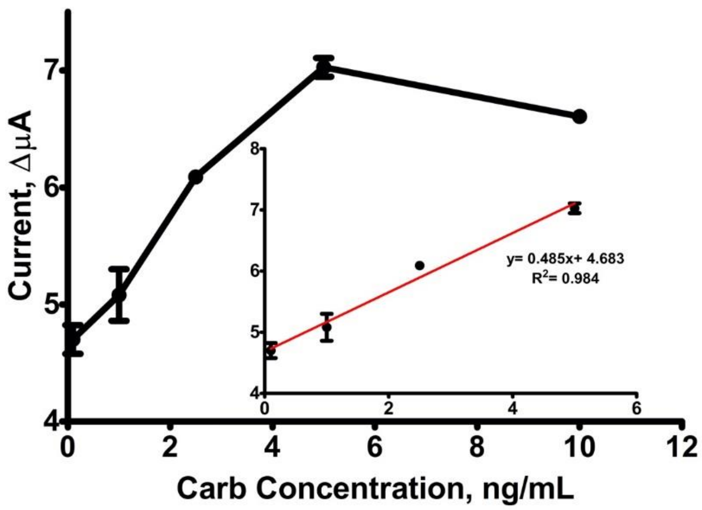


***Figure 11. Calibration curve of Gold electrode/Cys/Carb-17 Aptamer for Imidacloprid.***
*Generated by DPV technique, error bars shows ±SD.*

For determination of selectivity of the sensor, additional experiments were conducted with possible interfering molecules such as a 6-CN, thiamethoxam and thiacloprid. Interference molecules have responded on the sensor platform.

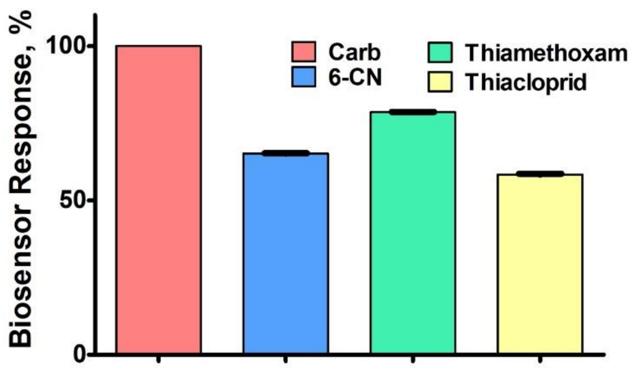


***Figure 12. Response of the Pirimicarb sensor for different interference molecules.***

## Evaluation of sensor performance in Use Cases

It was planned that the sensor would be designed as lateral flow or dipstick [12], and electrochemical biosensors would be designed as a second plan. Aptamer synthesis and improvement studies of aptamers have been carried out, and evaluation of their performance took a long time. Later, due to the coronavirus pandemic, the laboratories were closed for a while and the experiments were delayed. For these reasons, the electrochemical sensor platform, which is the second plan in the project, has been completed as a work package of Ege University. Therefore, a method was developed with screen-printed electrodes for *on-site analysis* of water samples. For imi detection, screen-printed gold electrodes (SPGE) were used because of their interactions with thiol groups in the aptamers. For carb detection in the water samples, the 3-electrode system was used. These systems were evaluated for two use cases in Sweden and Turkey.

### *Sweden Use Case*

**Imidacloprid**

Five wastewater samples (S1, S2, S3, S4 and S5) from the Sweden use case region were analysed for imidacloprid using the SPGE (see D2.6). Calibration of the sensor gave a linear response between 0.05-1.0 ng/mL (Table 5.). The values obtained for the water samples are given in Table 6. The response from the wastewater samples appeared below the value in the linear range. According to the responses received from the sensor, no pesticides were found in the wastewater. Then, water samples were examined by chromatographic analysis to ensure the accuracy of the sensor. It was observed that there were no pesticides in wastewater.

***Table 5. Signal responses of the Imi-sensor during calibration.***

| Concentration (ng/mL) | Current Signal (ΔμA) |
|---|---|
| **0.05** | 3.71 |
| **0.1** | 7.70 |
| **0.5** | 12.33 |
| **1.0** | 17.25 |

***Table 6. Signal responses of the sensor from wastewater.***

| Waste Water | Current Signal (ΔμA) |
|---|---|
| **S1** | 2.48 |
| **S2** | 1.33 |
| **S3** | 2.32 |
| **S4** | 3.11 |
| **S5** | 2.89 |

### *Turkey Use Case*

Three wastewater samples (T1, Hotamıs and Konya-Eregli) from the Konya use case region were analyzed using a previously designed and optimized sensor system. Screen-printed gold electrodes (SPGE) were used for detection of imidacloprid (Imi) and gold electrodes (GE) for pirimicarb (Carb) (see D6.4).

**Imidacloprid**

Through calibration a linear range was obtained between 0.05-1.0 ng/mL (Table 7). The values for Imi obtained from wastewater are given in Table 8.

***Table 7. Calibration of the Imi-sensor.***

| Concentration (ng/mL) | Current Signal (ΔµA) |
|---|---|
| **0.05** | 3.71 |
| **0.1** | 7.70 |
| **0.5** | 12.33 |
| **1.0** | 17.25 |

***Table 8. Signal responses of the sensor from wastewater.***

| Waste Water | Current Signal (ΔµA) |
|---|---|
| **Konya-Eregli** | 2.13 |
| **T1** | 1.87 |
| **Hotamıs** | 1.93 |

**Pirimicarb**

The linear range obtained for Carb in the sensor system was between 0.1-5.0 ng/mL (Table 9). The values for Carb obtained from wastewater samples are given in Table 10.

***Table 9. Calibration of the Carb-sensor.***

| Concentration (ng/mL) | Current Signal (ΔµA) |
|---|---|
| **0.1** | 4.03 |
| **1.0** | 5.08 |
| **2.5** | 6.09 |
| **5.0** | 7.03 |

***Table 10. Signal responses of the sensor from wastewater.***

| Waste Water | Current Signal (ΔµA) |
|---|---|
| **Konya-Eregli** | 1.93 |
| **T1** | 1.63 |
| **Hotamıs** | 1.57 |

### *Conclusion*

When the water samples were applied to sensor systems, the results obtained were below the detection limit. Even though the sensor platform designed for pirimicarb aptamer responded to the other pesticide molecules, Pirimicarb or any other pesticide could not be found in the waters when working with water samples from Konya. This indicates that Imi and Carb are either absent or not in the linear range of the sensor systems.

## Factsheet: Sensor for plant protection products

This factsheet gives data of the performance of the AgriNuPes sensor for plant protection products based on the outcome of laboratory and brief practical testing. Data should be considered as preliminary, as extensive testing under real-practical conditions have not been performed.

***Table 5. Description of the electrochemical biosensor for imidacloprid and pirimicarb***

| | Description |
|---|---|
| What | Biosensor for direct measurement of plant protection products, based on the electrochemical biosensor technology.<br>Available as an on-site test kit with active selective compounds for detection of pirimicarb and imidacloprid.<br>Exhibiting successful characteristics in terms of applicability, reliability and stability during field application. |
| Features | • Biosensor with disposable electrode technology for direct measurement of pirimicarb or imidacloprid (not yet available as dipstick).<br>• Working range suitable for mentioned use cases.<br>• Device with lifetime for > 1 year.<br>• High accuracy to discriminate legislative norms.<br>• Low temperature and pH sensitivity (not yet tested).<br>• Low cost for device and disposables. |
| Boundary conditions | *To be defined* |
| Operating conditions<br>- Temperature range for accurate measurement<br>- Measuring time<br>- Time before reading the sensor | *To be defined* |
| Target Audience | Technology suppliers, Farmers, Greenhouse growers, Nurseries Technicians, Advisory Services, Water Authorities, Scientist/Researchers |
| Accessibility | Contact EGE Life Sciences, Department of Biochemistry, Faculty of Science, Ege University, 35100 Izmir, Turkey (EGE-LS). |
| Disclaimer | This factsheet contains preliminary information and no rights can be deduced from the given specifications. |

*Table 6. Detailed specifications of the electrochemical biosensor*

| Specification | Target | Actual |
|---|---|---|
| **Measuring ranges** | range of concentrations | |
| imidacloprid | 0-5 µg/l | *1-50 ng/mL* |
| pirimicarb | 0-5 µg/l | *0.1-5 ng/mL* |
| **Operating conditions** | | water sample to be taken from nutrient solution contains ions, organic matter and PPP |
| Ballast ions | no influence of inorganic ions | |
| Organic compounds (PPP) | no influence of other (groups of) PPP | |
| Other chemical compounds | no influence of organic matter (no adhesion to sensor) | |
| **Performance** | | *Not yet available.* |
| Accuracy | 0.01µg/l | |
| Response time | <30s | |
| Drift | <0.5 µg/l | |
| Cross-sensitivity | Similar chemical groups | |
| Repeatability | <0.3 µg/l | |
| Lifetime | device for > 1 year; dipstick: disposable paper | |
| Temperature influence | no influence between 10-30 °C | |
| pH influence | no influence at pH 5-7 | |
| Selectivity | no other PPP within same chemical group | |
| **Housing** | | *Give the sensor a nice, practical box to avoid go missing and to keep dipsticks Not yet available.* |
| Materials: | - | |
| Resistant to: | pH 3-8 | |
| Dimensions: | 5 x 1 $cm^2$ | |
| Falling: | Resistant to 1x falling | |
| Read-out | Readable from 30-50cm | |

# 4. Usability and Applications

## Exploitable products

The AGRINUPES project delivered following exploitable products:

- NPK-Sensor (INESC TEC) consisting of:
    - a flow-cell, UV-light source and optical readout hardware (INESC TEC),
    - LABView (National Instruments) test platform for PC (INESC TEC),
    - Optional multi-variate calibration routine (INESC TEC)*.
- Control algorithm (software) / Embedded control algorithm (hardware) (INESC TEC)*.
- Fertigation controller with possibility of integrating NPK optical sensor (RITEC)*.
- Biosensor for imidacloprid and pirimicarb (EGE).

These products are described in Section 2 and 3, yet excluding the marked (*) items. The potential end-users of the products are growers, governmental organisations, technology suppliers and scientists. Their use can be defined as:

**Growers:** With these sensors, growers will have information about the input and output water quality and can evidence-based decide on how and when to irrigate and fertigate, and on whether the costly task of cleaning their irrigation water is advisable before disposal or appropriate to recirculate or not.

**Governmental organizations:** Water authorities may use sensors for checking water quality (pesticides) in ground and surface waters.

**Technology suppliers:** Agriculture suppliers of technologies and systems for water and nutrient management (fertigation controllers, water cleaning systems) can implement the sensors additional to their existing systems for feedback control applications. Resellers of equipment for agricultural practices world-wide can acquire a license to sell the sensors and decision support systems.

**Scientists:** Universities and research groups may want to use the (prototype) systems to further perform research on either the sensor's technologies itself or their application in agricultural set-ups.

## Best Management Practices

Along with validation of sensors, in several practical case studies, the Best Management Practices (BMP) for their use were developed. These BMP are described in more detail below in this section [including cropping systems, water type and usage (*e.g.*, influent, effluent, recycling), number of sensors, frequency and timing of measurements]. The following list gives a summary of identified BMP and refer to BMP-sheets in Case Studies descriptions.

- Real-time feedback in control loops of fertiliser dosing equipment (NPK-Sensor)
- Monitoring quality of water flows for decision support (NPK/PPP-sensor).
    - Disposal water in (greenhouse) horticulture (NPK/PPP-sensor).
    - Low-quality nutrient rich wastewater as input for re-use (NPK-sensor).
- Checking water quality of surface and groundwater (NPK/PPP-sensor).

## NPK-Sensors for feedback in control loops of fertiliser dosing (fertigation) equipment

### *Applications*

In high-tech greenhouse production (Use Case Spain, Netherlands, Sweden, Portugal).

The optimization of any fertigation system relies in the accurate measurement of the nutrient concentration in real time. For this purpose, it is mandatory to have a reliable sensing system capable of rapid measurement of the concentration of each relevant species to provide timely feedback. Such sensors should be able to function in a diversity of operational environments, sometimes in harsh conditions, depending on the type of crops. This requires for a robust technology able to operate in wet conditions without suffering from corrosion, and a modular approach where the sensor performance can be adapted to different ranges of concentration which will depend on the type of crop and point of operation for each system. AGRINUPES relies on an optical sensor technology to respond to this challenge.

***Figure 13. Potential application of NPK-sensors in fertigation systems for greenhouses.***

*Photo: Geomations, Athens, Greece; Spagnol, Italy; WUR-Glastuinbouw, Bleiswijk (NL).*

### *Benefits*

The NPK-sensors can be applied in irrigated crop-regions with a direct threat of restricted availability of water amount or quality. These are *e.g.*, the European (semi-) arid and wet regions with challenges connected to irrigation of horticultural crops: water quality, nutrition control and environmental issues. Especially regions with existing or upcoming environmental legislative constraints that do not allow contaminated water flows and thus force growers to change over to new practices. The solution to some of the issues could be to move from soil to substrate, from non-protected towards protected cultivation and from free drainage towards recirculation; however, there are still issues to be considered. Furthermore, the availability of good quality water is decreasing, and the use of alternative water sources is increasing. In these circumstances the main benefits of using the NPK-sensors are:

**Increasing the efficiency and resilience of water uses:** The sensors provide reliable measurements for online feedback control with less interference, low maintenance, and no need for recalibration. Thus, it can be integrated in the proposed innovative robust optimal control strategy and it will enhance the efficiency and safety of systems with wastewater reuse and recycling.

**Monitoring and reducing soil and water pollution:** the proposed control structure optimizes fertiliser application according to the actual nutrient concentration in the drainage water, thus reducing NPK losses to surface water and groundwater, since maximum recirculation of drainage water is made possible. In soil cultivations, feedback on leaching of nitrogen, phosphorus and potassium will improve the fertigation, thus increasing resource use efficiency and reducing losses and environmental impact.

## Monitoring quality of water flows for decision support (NPK/PPP-sensor).

### *Applications*

There are several options to check for water quality:

- Monitoring quality of disposal water or other water streams in (greenhouse) horticulture (NPK/PPP-sensor). Use Cases in the Netherlands, Sweden, Spain, Portugal.
- Monitoring of low-quality nutrient rich wastewater as input for re-use (NPK-sensor) in a second crop in cascade reuse systems (Use Case in Portugal).

***Figure 14. Discharge of drainage water from a substrate grown crop (The Netherlands).***

***Figure 15. A plastic-tunnel strawberry crop (Jordan).***

The above figure shows a typical production system where NPK-sensors could be used to monitor discharge, or the output water to be meant to reuse it in a cascade reuse system. In situations, like in Jordan, where primary crop is using nutrient rich treated wastewater, NPK-sensors can be used to measure the incoming nutrients concentration in order to adapt the fertigation mixture.

### *Benefits*

The main benefits of using the NPK or biosensors are:

**Increasing the efficiency and resilience of water uses:** The sensors will enable growers to check the wastewater and see if it is suitable to recirculate or whether it has to be cleaned or filtered in order to be recirculated, re-used or disposed. It will also enable to check the performance of their water cleaning equipment and mend possible malfunctioning to avoid hazards like unwanted emission of PPP or NPK. It will enhance the efficiency and safety of equipment to re-use of water and nutrients. By pre-checking water quality before cleaning, in those situations that cleaning is not required, growers can save on cleaning cost by not cleaning or cleaning at lower intensity or duration (energy cost). While reusing nutrients and water, cost for fertiliser use can be reduced. While reusing the water, growers become lesser dependent of the availability of water.

***Figure 16. Checking water quality in drainage water (Portugal).***

**Monitoring and reducing soil and water pollution:** By reusing water and fertilisers, the environment is not polluted with those fertilisers. Also, it is made possible to check the water for the specific PPP and avoid so the discharge of those chemicals. Growers might be able to state that they act responsible by "saving the environment".

## Checking water quality of surface and groundwater (NPK/PPP-sensor).

### *Applications*

Typical applications are found in the Use Cases in the Netherlands (greenhouses) and Turkey (arable farming).

***Figure 17. Continuous water quality monitoring (EC) and water sampling for lab-analysis.***

*Photo taken in Westland region (NL) during daily routine action from the "Hoogheemraadschap Delfland" (Delft, NL).*

For crop growing systems, either outdoor (open field agriculture) or indoor (greenhouses), it will in future become of utmost importance to comply with the Water Framework Directive and to not emit plant protection products. Having available the NPK/PPP biosensors, organizations like water authorities may use them for on-the-spot checking water quality (nitrate, imidacloprid and pirimicarb) in ground and surface waters. It will enable these organizations not only to respond more quickly but also to survey a much larger area at lower cost. Nevertheless, checking can only be done on sample basis, and not on the spot where potential hazards occur. However, as checking afterwards does not solve the issue of preventing pollution, the use of sensors to monitor on the spot the actual performance of grower's practices to prevent discharge of hazardous chemicals and pollutants, could also be performed by the growers themselves. By having available cheap and easy to use equipment (sensors), they could perform self-check on or around their production sites. As all agricultural fertigation water (discharges, or water streams from leakages) will include nitrate, a nitrate sensor can be used as an indicator for leakages. It can do so far better *e.g.*, than a common EC-meter, as those will not work in situations with higher NaCl concentration in surface waters. The AGRINUPES NPK-sensor could be used in a continuous mode to check at a strategic point (growers or water authorities). For growers however, it would be much better to have an easy to use and cheap handheld nitrate monitoring device. There are already such nitrate handheld metering systems on the market, but those tend to vary in their performance.

### *Benefits*

**Monitoring and reducing soil and water pollution:** Monitoring surface water quality, either on a sample basis or continuously, will not by itself prevent hazards, but rather detect hazards or dangerous trends towards environmental pollution. As such these methods can be used for checking and advising growers on their practises. On the long-term, grower's behaviour could be altered in order to achieve good water quality in a certain area. Experience of over a decade with this method in The Netherlands has shown that working together on an equal basis with growers seems more beneficial than maintaining and enforcing legislation.

***Figure 18. Checking for possible leakages in the nutrient dosing and irrigation systems.***

**Increasing the efficiency and resilience of water uses:** Checking water quality of surface and groundwater does not directly increase efficiency of grower's practises nor does it make these practices more resilient to water scarcity. However, once a grower has altered his practices in such a way that it complies with regulations, monitoring of surface waters can be done to monitoring that system and adequality act to mend any sudden failures. In that way this technology can be used indirectly to maintain a good practise and make it more durable.

# 5. Case Study Examples

This chapter describes for several European regions, possible applications for typical agricultural practises. Within these cases, the best practises described in the previous chapter, will be pointed out if relevant for the specific case. The following cases are defined:

- South-East Spain: Soilless growing systems in greenhouses.
- Portugal: Cascase ReUse systems.
- Turkey-Konya closed basin: open field agriculture.
- South-Sweden: soil grown vegetables, fruit, berries and ornamentals in greenhouses.
- The Netherlands-Westland region: High-tech greenhouse production.

## Case Region: South-East Spain

### Introduction:

The Murcia region in Spain lies in the Segura River Basin. It is located in the South-Eastern Spain, which is considered the most profitable agricultural semi-arid region of Spain. Scarcity of fresh water for irrigation is the main factor limiting crop production, motivating the use of reclaimed water, but usually it is of low quality (mainly saline) and besides there is over application of fertilisers. The idea of this case study is to use the NPK-monitoring system to support recycling of drainage water in greenhouse substrate production.


***Figure 19. Segura River Basin (Murcia, Spain).***
*Source: Google Maps (Nov. 2020).*

### Crop/cropping system:

The main crops grown in greenhouses are tomatoes (2,396 hectares in Aguilas, Mazarrón and Lorca), peppers (1,220 hectares in Torre Pacheco and San Javier), table grapes (919 hectares in the Guadalentín Valley, Molina, Las Torres de Cotillas and Abarán), courgettes (280 hectares distributed across the Region) and flowers (210 hectares in Cehehín and Puerto Lumbreras). Fruit trees, such as cherry trees and papaya trees, are also gaining ground in this sector. Crops are mostly grown in plastic type greenhouses, in forms like chapel greenhouses, sawtooth, Venlo or Dutch type, Almeria type greenhouses, mini tunnels or multi tunnels. The greenhouses have automated air conditioning and automated fertigation systems (Freshplaza.com).

***Figure 20. Tomatoes grown on soilless growing systems in plastic covered greenhouse.***

## Description (facts and figures):

In Spain the agricultural sector uses approximately 75% of the total water resources for irrigation. Sixty percent of the total production comes from just 20% of irrigated crop land in the Segura River Basin (Murcia). It is located in the South-Eastern Spain, which is considered the most profitable agricultural semi-arid region of Spain and has an estimated area of 6,235 ha of plastic covered greenhouses. The Murcia regions ranks second on the Spanish mainland for the acreage of vegetable production in greenhouses. Only Almeria with 31,931 ha is larger (Freshplaza.com).

Scarcity of fresh water for irrigation is the main factor limiting crop production, motivating the use of reclaimed water, but usually it is of low quality (mainly saline) and besides there is over application of fertilisers. The idea of this case study is to use the NPK-monitoring system to support recycling of drainage water in greenhouse substrate production.

**Implementation:** The NPK-sensors can be useful in the Murcia case study. The NPK sensors can be connected to a NutriTec irrigation and fertigation unit (see Figure) from RiTec (Spain) and integrated into a mixing-unit control loop. The integration is described in D4.4 “Integration module with nutrition unit, according to new sensors specifications for on detection of ion selected fertilizers”.


***Figure 21. NutriTec fertigation unit (RITEC).***

Ritec prepared the design (see Figure 22) of the controller equipment in a way that the new sensor could be installed easily on the structure with a straight access to the nutrient solution flow. In that way, the sensor can be measuring in real time the correct mixture of the nutrient solution. The best location to insert the sensors was evaluated. When a tank for the nutritive solution is not available, the sensor can be inserted in the pipe or a collector.

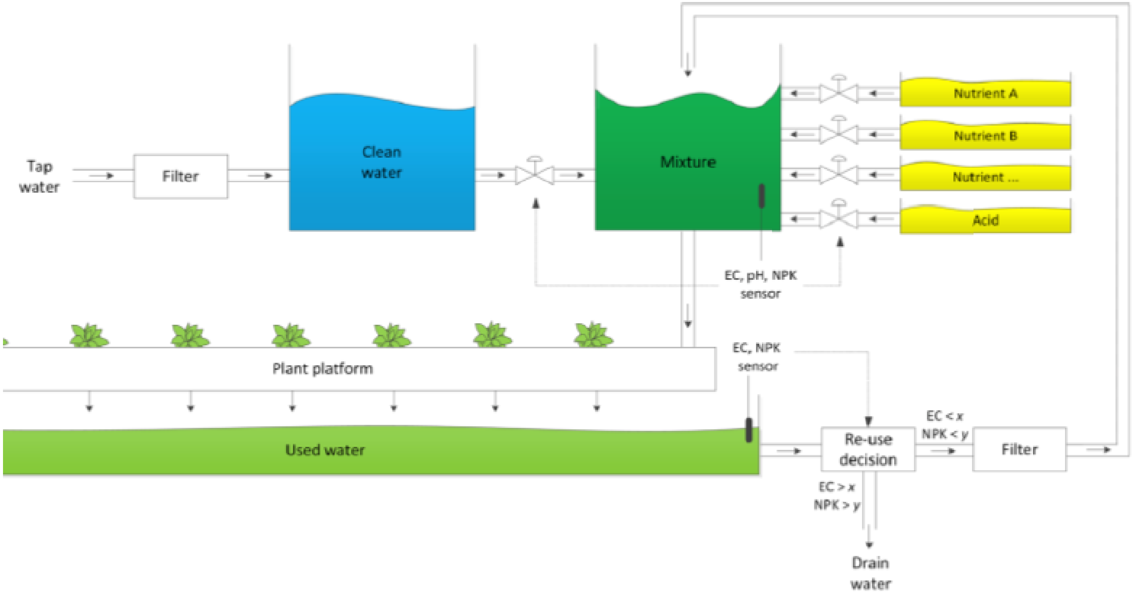


*Figure 22. Schematic design of the fertigation unit and the water system (RITEC).*

## Case Region: Porto – Portugal

### Introduction

In 2019, Portugal was ranked 14th in terms of cultivated area of horticultural crops in the EU, with 48.4 thousand hectares (considering fresh vegetables and strawberry), corresponding to a total production of more than 2 million tonnes [34,35].

Currently, the total greenhouse production area in Portugal is estimated to be about 3,000 ha, with a tendency for expansion. Amongst the most important greenhouse crops are tomato, bell pepper, leafy vegetables (*e.g.*, lettuce), strawberry and several ornamentals (*e.g.*, cut flowers) (INE, 2019). Protected cultivation in Portugal modernized in recent decades but remains heterogeneous in terms of technology, yields and management. The sector organizational structure and technological trajectory is characterized by increased greenhouse area per grower and higher volume/covered area ratio and increasing implementation of soilless cultivation. Therefore, less expensive alternatives may arise as the common solution for the sector. Most of the greenhouse area (about 75%) corresponds to single and multiple plastic tunnels with semi-automatic or automatic climate control. The use of glasshouses with fully controlled environment remains minor, due to the high costs of equipment, energy consumption, and limited expertise and technical support for local conditions [24].

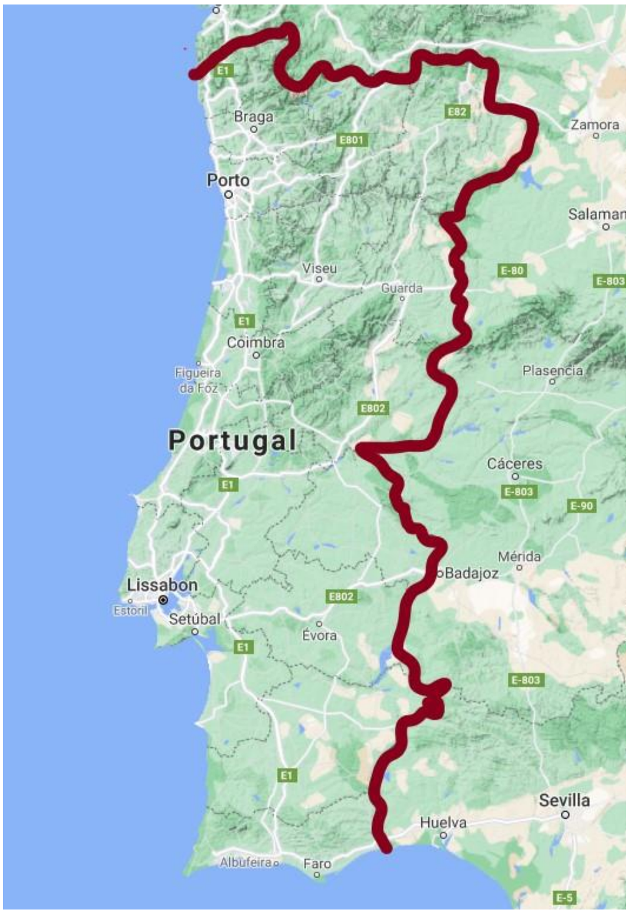


***Figure 23. Portugal.***
*Source Google Maps (Nov. 2020).*

Despite greenhouse cultivation is generally recognized as a more efficient production method when compared to open-field conditions, it can have undesirable environmental impact, particularly in non-recycling production systems. Therefore, greenhouse production must be properly monitored and optimized, in order to assure its socio-economic benefits and environmental sustainability. In addition, there is stricter environmental EU legislation and stakeholders are more informed which puts pressure on the greenhouse industry to be more efficient, thus becoming more sustainable. However, the Portuguese horticultural sector still lacks relevant statistics and standards on performance indicators, such as resource use efficiency (*e.g.*, water, energy) and related economic/environmental performance.

***Figure 24. Greenhouses (plastic) in Portugal with water reservoirs.***

## Description (fact and figures)

In Portugal, protected horticulture represents only 4% of the total area (75,000 ha) in which horticultural crops are grown. Despite most of the protected cultivation is still performed in soil, the area of soilless cultivation has been increasing in the last years, representing, nowadays, an important part in the Portuguese horticultural sector. Agriculture consumes up to 80% of the water consumed in Portugal and horticultural crops are of particular importance in this respect, as they are highly demanding in water, generally.

Concerning soilless cultivation, free drainage (open systems) and semi-open systems (so called Cascade ReUse Systems; “CRU”) are still the most utilized by Portuguese growers. The former is by far the most inefficient, since the drained nutrient solutions are totally lost, without any use, thus representing a huge environmental burden. In semi-open systems, drainage is reused for fertigation of other crops (secondary crops), typically of lesser economic importance for the grower and most commonly grown on soil, in land parcels contiguous to the greenhouse in which the soilless crop is installed (main crop). Despite this utilization of drainages, it is often only partial, because of limitations in retaining large amounts of drainage coming from the main crop and insufficient land available to apply those surpluses. Furthermore, environmental sustainability of semi-open systems has been questioned due to uncertainties relative to the effect of long-term application of drainage to agricultural soils and to the risk of deposition of PPP and high levels of nutrients into water bodies in the vicinity of the production areas. Therefore, increasing the implementation of closed systems is crucial in achieving satisfactory use efficiencies of water and nutrients and improving the environmental performance in soilless horticulture. In Portugal, as well as in other important horticultural regions, generalization of using closed systems has been limited by several factors, being higher investments and running costs, along with higher need for technical knowledge, amongst the most decisive. In this respect, the use of smart monitoring systems, which are also cost-effective, reliable and easy to use, are of the utmost importance in supporting fertigation management.

## Crop/cropping system

In the case of CRU, the most common situation in Portugal is that growers use the drainage originated from the cultivation of soilless crops, for fertigation of soil-grown crops, either in open-field or in another greenhouse [25]. In brief, the nutrient solutions are applied to the main crop (soilless) in excess, as a common practice, and the resulting drainages are retained in reservoirs or ponds, to be later used for fertigation of secondary crops (Figure 25).

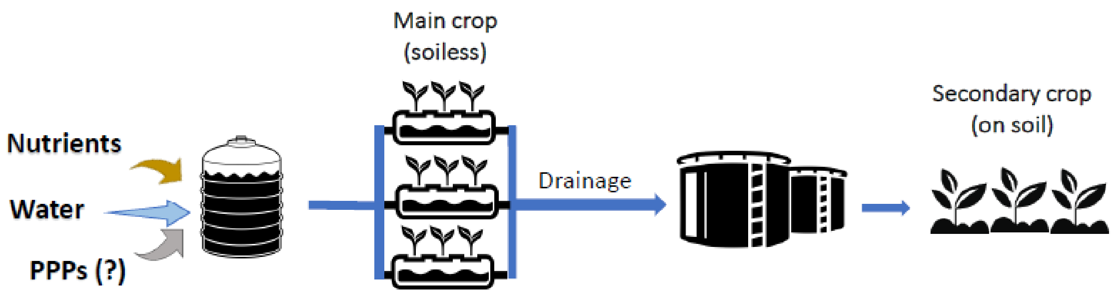


***Figure 25. Schematic representation of a Cascade ReUse System.***

Despite that growers perceive the amount of drainage resulting from soilless cultivation as an enormous quantity of water and nutrients, open systems still represent an important part of the production systems adopted by Portuguese growers. In order to characterize the Portuguese soilless cultivation sector, particularly in terms of irrigation management and application of PPP, we applied, in 2017, a questionnaire to Portuguese growers from two on the main horticultural regions [24]. This questionnaire allowed to obtain the following conclusions (for further information, refer to D5.1):

- There is a low use of drainages, as open systems are very common (42% open systems; 42% CRU; 16% closed systems);
- Irrigation needs are frequently evaluated empirically (75%), by drainage volume (58%), radiation (17%) or temperature (17%);
- More than half of the growers (58%) control the drainage, and the parameters most commonly monitored in these solutions are EC (100%), pH (71%) and volume (43%);
- The reasons for not adopting a closed system are investment cost (100%), lack of technical knowledge (29%) and other reasons (43%), such as lack of confidence on disinfection methods;
- There is a clear need for economical and expedite technologies to support decision making, regarding fertigation management.

After this general characterization, AGRINUPES performed an assessment for the potential for reusing the drainages and for the suitability of using CRU, particularly those including soil cultivation. Drainage samples from three Portuguese commercial CRU were collected in two time points (autumn-winter and spring-summer periods), considering the distinct fertigation management commonly applied in different stages of the cropping cycles. These samples were analysed for their nutrient content, PPP residues and ecotoxicological assays were performed using aquatic species, including green microalgae, microcrustaceans and bioluminescent bacteria, which were exposed to drainages following standard protocols (*e.g.*, ISO, OECD). From the characterization of the drainages, it was possible to conclude that they carry large amounts of main macronutrients (N: 51 – 460 mg/L, P: 1 – 43 mg/L, K: 12 – 854 mg/L) and that, in overall, the drainages from the tomato CRU were the ones with highest nutrient content, followed by the drainages from the rose CRU and strawberry CRU.

While prevalence of high concentration of nutrients in the drainages indicated good potential for their use in crop cultivation, the different pattern of nutrient consumption constitutes a challenge for recirculation, thus highlighting the importance of application of sensing systems in fertigation management. Furthermore, the concentration of N and P of the drainages from all CRU were compared with the Emission Limit Values (ELV) according to Portuguese legislation, in order to emphasize what drainage emission to the environment may represent. For N (ELV = 15 mg/L) drainages largely exceeded the limit, being up to 31-fold higher in drainages from tomato CRU, 4-fold higher in drainages from rose CRU and 3-fold higher in drainages from strawberry CRU. For P (ELV=10 mg/L), despite to a lesser extent, the threshold was also exceeded, ranging from 1.5 and 3.5-fold higher than the ELV. There are no specific thresholds for K, but for total metals (ELV = 10 mg/L) in some regions (e.g., Lisbon Municipality), and the drainages showed K concentrations above that limit as well (ranging from 7 to 77-fold higher).

Regarding the irrigation water quality, it was possible to conclude that PPP residues tend to persist in the production systems since, in general, similar quantities of the same PPP's active ingredients were found in drainages collected from the reservoirs (*e.g.*, where drainages are retained) as compared to the PPP residues found in the nutrient solutions immediately drained through the growing media. From 15 analysed PPP on drainages collected from reservoirs, 10 were detected for rose CRU, 8 for strawberry CRU and 2 for tomato CRU. For example, we found dimethoate (36.1 µg/L, rose CRU; 36.2 µg/L, strawberry CRUs); boscalid (23.9 µg/L, tomato CRU).

***Figure 26. Strawberry grown in mix of different substrates (left). Rose on coco peat (right).***

The impact of using CRU was also studied in terms of soil quality, since the majority of these systems use the drainages in cultivation of soil-grown crops. Based on the results from the assessment of drainage quality in previous tasks, the strawberry CRU was considered a worst case scenario and selected as case study. Soil samples were collected from a greenhouse in which drainage has been used for about five years in fertigation of soil-grown crops, and two reference soils were collected for comparison. One of these reference soils was from the inside of the greenhouse, but not receiving drainages, and the other reference soil was from the outside of the greenhouse and considered as representative of the location. Soil quality was assessed in terms of retention function for nutrients and PPP, soil habitat function and soil fertility. Retention function was assessed through ecotoxicological assays using aquatic species (same as for drainages) exposed to different concentrations of soil elutriates and through quantification of PPP residues in those soils. Soil habitat function was assessed through reproduction tests using important soil bioindicators, namely earthworms and springtails. Soil fertility was assessed through the activity of soil enzymes involved in nutrient cycling and through a plant growth test. It was seen that CRU represent an environmental hazard, as long-term application of drainages to agricultural soils can be harmful to aquatic organisms living in water bodies receiving drainage run-offs and can also cause algae proliferation, that could ultimately lead to eutrophication of aquatic environments. The CRU affect the soil habitat function (both survival and reproduction of soil organisms) and fertility due to soil salinization, deposition of PPP residues and impact on nutrient cycling.

For assessing the impact of CRU on a secondary crop two greenhouse trials were conducted in soil (used in previous task) and hydroponics, using lettuce as model crop and fertigation consisting in different proportions of drainage incorporated in the nutrient solution (Fig. 26). Plant performance in both production systems was assessed in terms of growth and leaf mineral composition, including leaf nitrate concentration, which is of particular importance in leafy vegetables. In hydroponics, growth was not affected using drainage percentages of 25% and 50%. Using 100% drainage, decreased head size (-20%), leaf area (-41%) and fresh weight (-35%) were observed. In the soil trial no significant differences on growth were observed among treatments, probably because of slower plant development observed in this production system, which typically requires much more time for crops to reach an adequate size for harvest, as compared with hydroponic conditions. These trials allowed to conclude that there is a good perspective for using high percentages of drainages (*e.g.,* 50%) in closed system or in cascade cropping systems consisting in soilless cultivation only, as drainage application to soils contribute to their degradation, thus indicating that soil cultivation should be avoided in CRU. In addition, we highlighted the importance of producing guidelines and monitoring in the use of drainage for crop fertigation.

***Figure 27. Trials with a secondary crop (lettuce) in soil (left) and a hydroponic system (right).***

## Implementation

The sensors were implemented in a case study in Porto for Cascaded Re-Use system in close collaboration with local growers and grower organizations. In their growing system, the drained nutrient solution from the soilless cultivation is normally collected but not reused in the main crop. Instead this is partly used to irrigate a secondary crop (in open field or in another greenhouse), with lower economical value.

The sensors will be installed in practice at a selection of growers with the most critical situations in terms of impact at water, soil and/or plant level. The performance of the NPK-sensors will be evaluated, the sensors will be demonstrated, and guidelines will be established for the development of strategies to define water quality parameters and thresholds for decision-making recommending possible adjustments to the current irrigation methods in this type of systems.

The field application of the NPK-sensor in the Portuguese Use Case is described in D5.3.

## Best Management Practice

The location to install the sensors in the irrigation water system is important. In principle the preference is to use the sensors both at the beginning of the irrigation system as well as at the end in the drainage water.

In case of an open field application, the NPK sensors must be installed at least at the beginning of the irrigation system (at the mixing unit), in the storage containers, or at the inlet position to the crop irrigation. In case the drainage water is accessible (*e.g.,* when drainage pipes are laid under the crop root-zone and have an outlet to a ditch, canal or similar, it is advised to position a sensor there as well. If the drainage water is not accessible, the NPK concentration can be obtained in the traditional way by taking soil samples and having them analysed by a lab.

In protected crop production systems, the sensors may be installed both at the beginning of the irrigation system as well as in the drainage water.

## Case Region: Konya Closed Basin, middle Anatolian region of Turkey

### Introduction

Konya Closed Basin located in Central Anatolia of Turkey is one of the hot spots of Turkey in terms of intense agricultural activity in a semi-arid climate resulting in over exploitation of natural water resources [29][30ng a closed basin, the region is hydrologically isolated, meaning that it heavily relies on its self-capacity to renew and protect its water potential. The combination of intense agricultural activity and being a closed basin necessitates cautious monitoring and control of agricultural inputs in order to maintain good ecological status in the basin. Taking this situation into account, the Konya Closed Basin was chosen for the field application of the sensor and decision support tools that are being developed. The Basin characteristics for field application of the AGRINUPES technologies are described in D6.1.

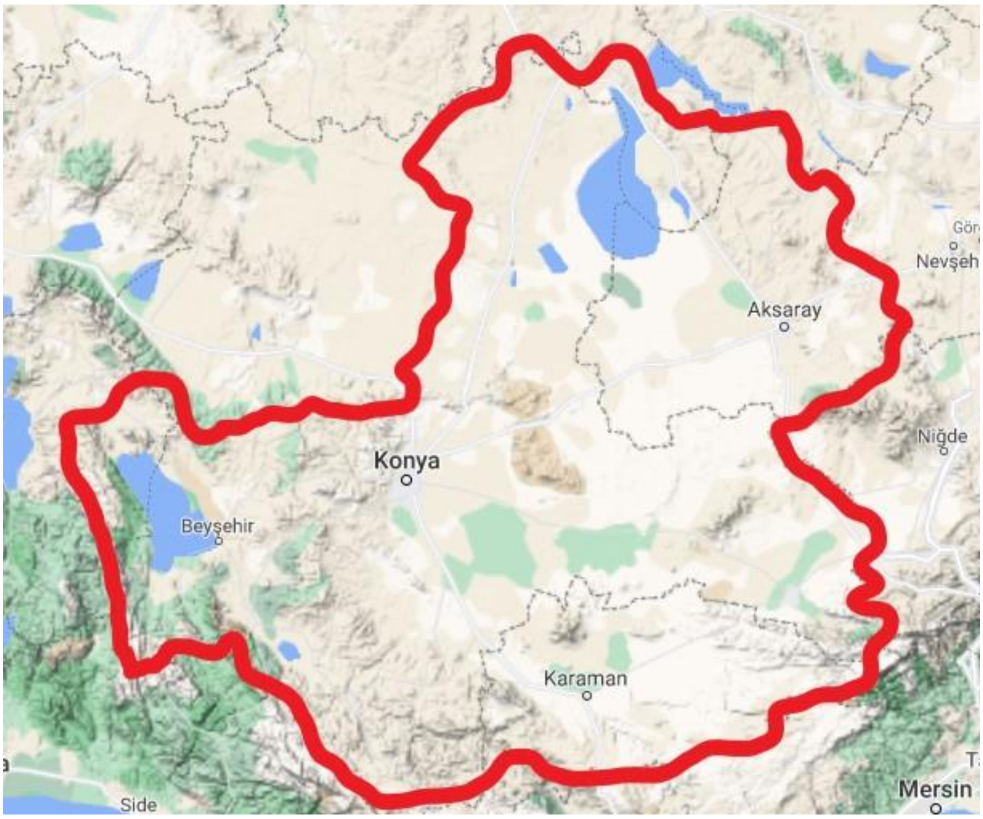


***Figure 28. Konya Closed Basin***
*(source: Google Maps, Nov. 2020)*

### Description (facts and figures)

Turkey has a total arable land of 25.7 Mha of which 17.2 Mha can be cultivated as rain-fed land, whereas the remaining 8.5 Mha are economically irrigable using available technology. At present, only 5 Mha are under irrigation, but the aim is to equip the whole area with irrigation facilities by 2023.

The Konya basin has a semi-arid climate condition. The total agricultural area is: 2.77 Mha which is about 56% of the total area. Crops grown are mainly sugar beet, potato, sunflower, wheat, barley, rye, oats, apple, pear, grape, and tomato. The average annual precipitation of the basin is 380 mm. This is almost half of the total averaged annual rainfall (740 mm) in Turkey, varying from 250 mm in flatter parts up to more than 1000 mm in mountainous areas. The precipitation amount is more in the south and southwest of the basin in comparison with northern and eastern parts [26]. The surface water holds about 2.4 $km^3$ of water, and the groundwater is about 2 $km^3$.

The winters are cold and wet while summers are hot and dry. The south western upstream part shows a warmer and rainy Mediterranean character, while the rest of the basin has a drier, continental climate, isolated from the moderating effect of the Mediterranean Sea by the Taurus Mountains in the south [26]. Annual average temperatures vary between -0.4°C and 23.0°C. July and August are the hottest months, while January and February the coldest months in the basin [28].

***Figure 29. Typical open filed agriculture in Turkey (Konya Basin).***

Despite being one of the most important agricultural and agro-industrial regions, the Konya basin is characterised as a water scant region. In recent years, the annual water demand (4.9 $km^3$/year) of the Konya Closed Basin exceeds the basin's total available water potential (4.43 $km^3$/year) which is caused by unplanned increase in number of irrigation areas, crop pattern preferences with high water consumption levels, lack of public awareness and inefficient irrigation applications.

A large part of water budget deficit is covered by groundwater reserves. Therefore, groundwater reserves are facing a depletion threat. This situation causes the basin to move away from environmental and agricultural sustainability.

The irrigated area is about 650,000 ha (sugar beet, potato, wheat, barley and sunflower), from which 115,000 ha correspond to sugar beet. Irrigation water is mainly obtained from ground water resources. The Konya Closed Basin is known to be the highest use of groundwater in Turkey [27], and there is an increasing awareness of the necessity to reduce contamination by plant protection chemicals and to invest in precision irrigation in order to minimize leaching of fertilisers (D6.1). The framework for regulation of nutrient and pesticides legislation are a little different in Turkey compared to Europe (D6.2), though with comparable threshold levels. A remarkable difference are thresholds for Imidacloprid (0.14 µg/L), as compared to The

Netherlands (0.0083 µg/L), and for Pirimicarb (3.3 µg/L) as compared to The Netherlands (0.09 µg/L).

### Crop/cropping system

The Konya Closed Basin is mainly characterized with open field agriculture. The main agricultural products cultivated are wheat, barley, rye, oats, sugar beet, sunflower, corn, potatoes, lentils, beans, pods, linen and hemp. Furthermore, some fruits and vegetables such as apple, pear, grape, tomato, pepper, aubergine and cabbage are also grown in the basin [28].

The agricultural areas in the basin are (still) irrigated by surface irrigation systems through open channel transmission lines. However, farmers tend to use groundwater from unlicensed wells, due to distribution problems with transmission lines and technical deficiencies in accessing irrigation water in times of need [26].

### Implementation

The Konya basin is chosen due to its exceptional case in the sense that farmers themselves already encounter water quality and quantity problems due to mismanagement of irrigation and fertilisation management. The utilization of AGRINUPES sensors were to be evaluated and demonstrated in open field with the active involvement of local farmers into monitoring practices/processes. The main aim was to evaluate the ability of the sensors to detect presence of nutrients in water resources close to agricultural fields and to provide guidelines for user-friendly, cost efficient and time saving measuring methods. A further aim was to comply with EU legislation and develop participatory policy guidelines for optimizing the use of nutrients and pesticides in line with the EU Water Framework Directive. More details are described in the report on current agricultural practices/policies and evaluation of the AGRINUPES tools' potential for implementing EU legislation (D6.2) as well as the Policy guideline report for the utilization of AGRINUPES sensors (D6.3).

### Results

As mentioned previously, Konya Closed Basin is the biggest user of groundwater in Turkey and there is an increasing awareness of the necessity to reduce contamination by PPP and to invest in precision irrigation in order to minimize leaching of fertilizers. Thus, the aim of this open-field study was to investigate the hindrances behind the optimum use of agricultural inputs in current agricultural practices, the bottlenecks in current water and agriculture related regulations and study the practicality of employing sensors for open-field applications.

Unfortunately, the field application of sensors could not be carried out as planned in the open field due to delays encountered in the development phase of sensors and Covid-19 outbreak right after. The open field study aimed not only to test sensors in open field, but also demonstrate the main working principles of both sensors to potential end-users (*e.g.*, farmers, local water and agricultural authorities) with the purpose of getting their feedback on their practicality. Since a field visit could not be carried out, samples were instead collected from designated agricultural discharge points of Konya and sent to Ege University for analysis at the laboratory. The results are given in detail in D6.4. Even though the field application could not be conducted as planned, the bottlenecks in current agricultural practices and policies are investigated both through desk research and by the formation of a User Network Group (UNG). A short survey has been conducted with the members of UNG (members consisting of local water and agriculture authorities, farmers) to get their feedback on current policies and also to get their initial opinions on the sensors. In brief, surveys revealed that there are problems in the implementation of policies as result of their complexity and ineffective enforcement. Yet the end-users trust that the development of innovative sensors would help abate the majority of the bottlenecks faced under current practices. More details can be found in the “Policy guideline report for the utilization of AGRINUPES sensors” (D6.3).

### Best Management Practice

A Best Management Practice for the use of the sensors in the Konya basin is described in the report D6.3: “Policy guideline report for the utilization of AGRINUPES sensors”.

## Case Region: Skåne in the southern part of Sweden

### Introduction

Greenhouse production in Sweden is a small but important business that has a politically intension of increasing. Approximately 60% of the greenhouse production area have recirculation of the irrigation water and there is a large part of the greenhouses that are old and thus are low-tech buildings.

Since 1992 a surveillance of pesticide residuals in surface waters in the environment is going on. In 2008 these measurements were complemented with measurements in greenhouse close areas. The results were clearly problematic showing high chemical residuals in surface waters and since then pesticide leakages from greenhouse production have been in focus. Several follow-up studies have been made showing the same problems from many areas.

Only limited surveillance can be made due to the large cost associated with chemical analysis and it is thus difficult for the growers to know which waters should not leave the greenhouse.

If robust and reliable sensors for chemical residuals could be developed, this would be a large advantage that could assist the grower's decision of when to clean the waters from pesticides.

The first step to get the greenhouses more sealed is to recirculate the irrigation water. To optimize recirculation and nutrient supply to the plants the nutrient levels of N, P and K are important to know in the return water. With robust and reliable sensors for nutrients recirculation of the irrigation water could be more precise and optimal for the plants making them more resistant for plant diseases and pests.

### Description (facts and figures)

The agricultural area in Sweden is 2,6 Mha with 13,000 ha in horticultural crops. Nearly 60,000 ha of agriculture are irrigated. The greenhouse sector consists of 744 companies with a total greenhouse area of 286 ha. Approximately half of the area is used for ornamental pot plant production and the other half for vegetables (tomatoes, cucumbers) or berries (mostly strawberry and raspberry).

The water used in greenhouses, orchards and in the field is coming from high quality surface water sources, ground water, tap water or captured rainwater.

The main part of Swedish greenhouse production areas is situated in the southern parts of Sweden. Swedish main concern regarding the NPK sensors is the ability to optimize the nutrient composition when recirculating water in greenhouse production.

![AGRINUPES logo]


***Figure 30. Greenhouses area in Skåne (Southern part of Sweden).***
*Source: Google Maps (Nov. 2020).*

In Sweden the focus lies on voluntary precautions based on information and obligatory and voluntary training campaigns with respect to safe handling and application of PPP to solve problems with diffuse leakage, because it is believed that excessively detailed legislation would not be efficient [32]. Sweden is considering installing measures to reduce PPP pollution in surface water coming from greenhouses [30]. Growers are aware of the situation and are taking measures to decrease these leakages. However, they need a tool to quantify their efforts by measuring the water for chemicals. But the present analysis of these are quite expensive and they need a quick, efficient and non-expensive method to measure this. The biosensors for imidacloprid would serve as a valuable indicator tool for this.

## Implementation

Use of new sensors will help the growers to adjust the nutrient supply and enables better surveillance of the water quality so that the growers can make precautions when they have indication that the water needs purification. New sensors can also be used in environmental surveillance of PPP residuals both in surface and groundwater. This is currently done by expensive chemical analysis.

## Case Region: The Westland region, the Netherlands

### Introduction

The Netherlands is well known by its market in high-tech glasshouses for the production of vegetables, flowers and pot plants. The Westland region [38], located close to the North Sea, is one of the regions with a high concentration of greenhouse production (Figure 31). Since the nineties, substrate-grown crops with recirculation of drain water is the common practice, but discharges and leaching occur regularly (on average 5-10% of the supply given to the plants [30]. There is an urgent need of complying with emission restrictions [36] and drastically reduce leaching of fertilisers and PPP. To cope with this, growers need to optimally dose nutrients and clean their discharge water (remove PPP). Water treatment technologies are available but are rather costly. Use of new sensors and controllers will help them to improve operational decisions in their water management to achieve a high-quality crop and cope as well with regulations.


***Figure 31. Westland region, the Netherlands (source Google Maps, Nov. 2020).***
*Grey squares are greenhouses; brown spots are houses and villages.*

### Description (facts and figures)

With 3,500 companies, the total area of Dutch greenhouses is somewhat less than 10,000 ha (0.5-40 ha in size and on average 2.8 ha) with both soil-bound and soilless grown crops. Vegetables are grown by 1216 companies on 5,330 ha, while the rest are mainly ornamentals (cut flowers and pot plants, on 3837 ha). Nursery stock production and fruit form a minor part (5%) of the total area. Greenhouses are high-tech glass (Figure 32) with computer controlled climatization. The majority of the crops is grown on a soilless culture (90% of the area). Gutter systems with stone wool slabs or containers with different substrate types (peat, coir, stone wool) are being used (Figure 33). Major vegetable crops, often grown in a high-wire cultivation system, are tomato (1690 ha), sweet pepper (1313 ha), cucumber (545 ha), strawberry (491 ha) and aubergine (128 ha). The major soilless-grown cut flowers are rose (250 ha), gerbera (163 ha), lilies (157 ha) and orchids (117 ha). Many kinds of pot plants (on floors or on tables) have another 2000 ha soilless grown [31,37]. About 10% of the crops are grown in soil [31], because an economically feasible soilless production system does not yet exist for those crops. This applies mainly for some cut-flowers, namely chrysanthemum (Figure 34), freesia, alstroemeria, lisianthus), leafy vegetables (lettuce types) (Figure 35) and radish and organically grown crops, which include mainly tomato, sweet pepper and cucumber (100 ha).

**Figure 32. High-Tech greenhouse in The Netherlands.**
*Characterized as houses with diffuse glass, with a gutter height of 7 m, rainwater collection (left) and rainwater collection tanks and drainage pipes to the ditch (right).*
*(Wageningen University and Research facility in Bleiswijk, NL)*

***Figure 33. Substrate grown crops in typical Dutch high-tech greenhouses.***
*Tomatoes (left) and a gerbera crop grown in containers on gutters (right).*

***Figure 34. Soil grown crops (Chrysanthemum) in typical Dutch greenhouses.***
*A recently planted Chrysanthemum crop (left) and during cropping with artificial light (right).*

***Figure 35. A soil-grown radish crop (left), red oak leaf lettuce hydroponically grown in nutrient film technique (NFT) on movable troughs (right).***

Among the Northwest European countries, The Netherlands has the highest concentration of greenhouses, as surrounding countries like Germany, Belgium, Sweden, Denmark and UK, have only 500–3,500 ha of greenhouses scattered over much larger areas. Consequently, the polluting effects/risks have a different scale [30].

In open soilless cultivation systems (Figure 36A) the discharged nutrient solution (30-40% of the volume supplied to the plants) is flowing to the surface water or into the sewage system. In the first situation smaller ditches might be polluted, in the latter case large canals might receive the discharged solution with, amongst others, nitrate, phosphates and PPPs. In The Netherlands an open system is not allowed anymore. In the closed soilless systems (Figure 36B) most of the drain water is reused and only 5-10% of the volume supplied to the plants is discharged. Discharge of nitrogen must be decreased to nearly zero by 2027, while the PPPs must be eliminated with purification by 95% already since 2018 [30].

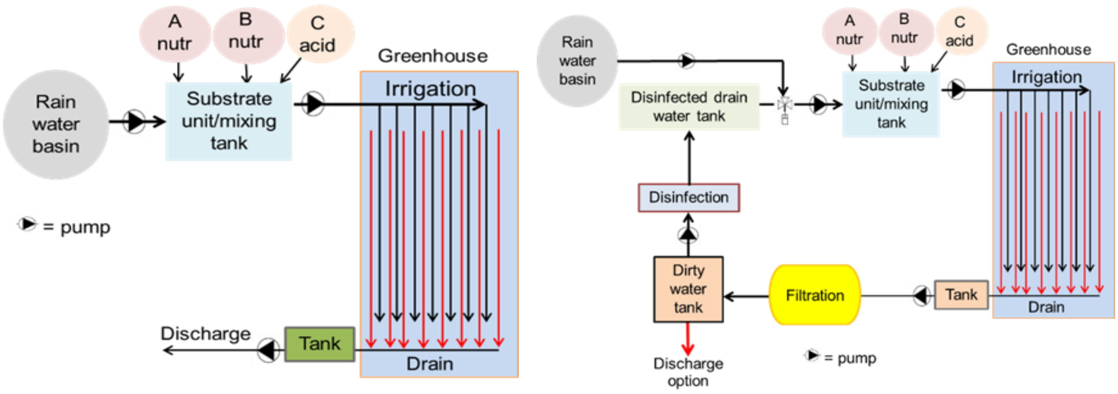


***Figure 36. Scheme of an open (A, left) and a closed (B, right) soilless culture system.***
*(Scheme used from Van der Salm et al.[30]).*

In soil systems the irrigated water seeps into the soil and if there is too much it drains away via drainpipes into the ditches around the greenhouse. Drainpipes are common in the Dutch polders where the ground water table is artificially maintained at 80-90 cm below surface level. Here there is infiltration and seepage of water depending the water table of surrounding canals and ditches.

## Quality of the surface water

In large parts of the Netherlands surface water quality does not meet the chemical and ecological standards as indicated by the EU Water Framework Directive (WFD). The largest exceedances were found amongst others in areas with greenhouse horticulture, mostly caused by intensive agriculture, shallow groundwater tables and intensive drainage systems. In 2014, concentrations for both nitrogen (N) and phosphorous (P) were exceeded in 45 % of the water bodies and exceedances of PPP were found at 60 % of the locations [30].

The current regulatory measure of the Dutch government to improve water quality in greenhouse areas is by achieving (nearly) zero emission of nutrients by 2027. It is assumed that a reduction in N emission will be mainly achieved by a reduction in the discharge of the water volume and will thus also reduce the emissions of P and PPP. Enforcement of regulation is assigned to the Water Authorities (regional semi-governmental bodies responsible for water quantity and quality).

For soilless cultivation, crop (and company) specific norms for the emission of N are defined, which will be gradually decreased until 2027. The nitrogen emission standard (2015) varies with crop type (9 categories) from 25 – 300 kg N $ha^{-1}$ $yr^{-1}$, with an estimated discharge volume of 100 – 3,600 $m^3ha^{-1}yr^{-1}$.

Since emission standards are not feasible for soil grown greenhouse crops, a different approach was chosen to minimise losses. Solutions were directed towards optimising irrigation and a sustainable use of fertilisers, together with regulations on crop-specific maximal nutrient usage.

In addition to that, an obligation exists to remove PPP from drain water by 2018 onwards (Figure 37). This regulation, the Purification Decree [39], based on an agreement between authorities and the growers' organisation, states that at least 95 % of the PPP must be removed from discharge water by using purification equipment [40]. This rule applies for both soilless and soil-bound greenhouse cultivation and for discharge to surface water and sewer systems. Van Ruijven *et al.*, 2020 [40] mention active ingredients as abamectin, boscalid, esfenvalerate, imidacloprid, kresoxim-methyl, pirimicarb, pymetrozine and spinosad as tested pilot substances for their environmental risks and representing various chemical groups.

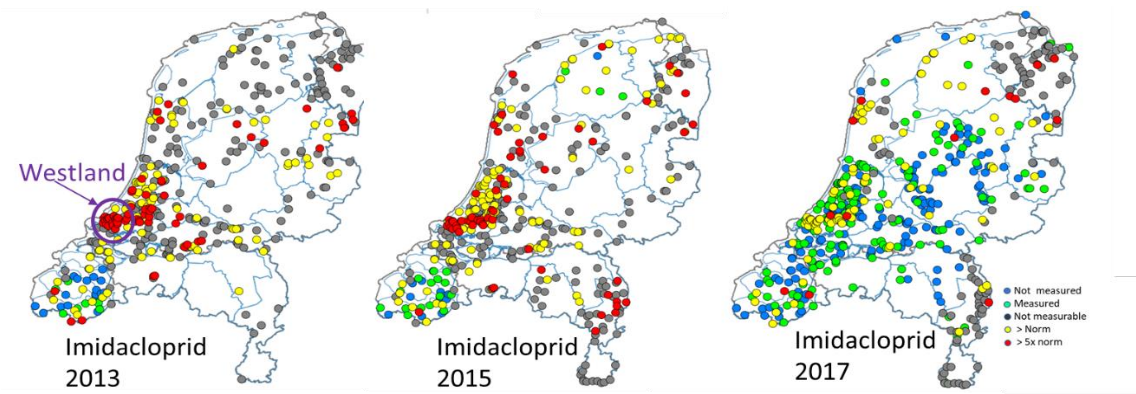


***Figure 37. Number of exceeding of norms for imidacloprid (2013, 2015, 2017).***

*Red dots >5x exceedance of the allowed norm, yellow dots 1-5x and green dots no exceedances of the norm (www.bestrijdingsmiddelenatlas.nl).*

Research to find appropriate practises for farmers to comply with these regulations showed that for soilless cultivation a Zero Liquid Discharge (ZLD) system gives the best option [40]. For soil-bound cultivation the situation is more complicated and a combination of tools and measurements to help the farmer to tune irrigation to crop demand is most promising [42]. These approaches will hopefully lead to a substantial decrease in discharge of nutrients and PPP to surface water, and, consequently to a better surface water quality as meant in the EU Water Framework Directive. Some progress can be seen in the reports of Delfland Water Authority in the Westland Region [43]. For some obstacles still solutions need to be found, as for problems with soil-bound cultivation, leakages in soilless cultivation and sodium limitations in certain crops.

## Implementation

### *Soil-grown crops*

An obvious solution for soil-grown crops would be to switch to a soilless culture. However, for various technical and economic reasons this is not always feasible. Most soil-grown crops have a relative short growing period (weeks or a few months) and a high planting density with almost full surface coverage. To achieve a similar setting in a soilless system, a yield increase of at least 15% would be needed to make soilless cultivation economically feasible. Since this is not possible for all crops, following measures are proposed for soil-grown crops [44, 30]:

- Reuse of drainage water
- Tuning irrigation to crop demand
- Tuning fertilisation to crop demand

Reuse of drainage water will drastically reduce the quantity of discharged drainage water and a strong improvement of the nutrient use efficiency. As the individual nutrient concentrations of the discharge waters is unknown and rather variable, the use of an in-line NPK-sensor would be beneficial to control the fertigation.

The most effective way to reduce leaching is to reduce the inputs of water and fertilisers, although a certain over-irrigation is the common strategy for soil grown crops to avoid salt accumulation. For many growers this can be a tricky approach as exact water need of the crop is usually not well known. To support these practises, a combination of Evapotranspiration (ET) and hydrological models, soil water content sensors or lysimeters are advised and investigated at this moment.

The concept of tuning fertilisation aimed at optimum production and quality has been used since the seventies and eighties in The Netherlands. There are possibilities to reduce N and P concentrations. The use of an in-line NPK monitoring technology could be useful to support this practise. Besides the irrigation and fertilization are more commonly done at the same moment to realise the right amount of nutrients during the entire growing period. In the past stock fertilization with organic manure or compost was much more important, while during cropping hardly any fertilizer was given.

### *Soilless cultures*

Emission reduction is relatively easy in soilless cultivation compared to soil grown crops, as drain water flows can be controlled. Dutch growers have fully climatized and computerised greenhouses, including measurement of global radiation and the irrigation is strictly related to the amount of (solar and artificial) radiation. However, as soilless systems have a small rooting volume, reducing irrigation can lead to severe problems. In practice the irrigation surplus can be reduced, but a drain fraction of at least 0.2 (vegetables: 0.3, flowers: 0.5) is recommended to prevent problems of heterogeneity in release of drippers, transpiration and uptake. The new legislation for emissions has forced growers to take up following practices [30]:

- Recirculation of drain water, to reduce emission of both nutrients and PPP.
- Purification of discharge water for the removal of PPP, to reduce emission of PPP.
- Zero Liquid Discharge (ZLD).

**Recirculation of drain water** has led to new infra-structures at the growers and is mandatory. Those systems are semi-closed as discharge is only allowed in rare occasions when the system fails to maintain a good quality water composition for production. Common practise is to control EC, pH and volume of irrigation continuously and to measure nutrient composition at least once per 2 weeks. Here is a possibility to use an on-line motoring system for the major nutrients (*e.g.* NPK). Still a tricky point is the leakage of nutrient solution. Within the greenhouse, water is lost by connections of troughs and pipes, drippers which are standing wrong or creating the first drain of the slabs. Each point has to be solved, growers has to be aware that between 0.5 and 1.5% of the volume applied is lost by leakages of the system.

**Purification of discharge water.** The water discharged from a semi-closed cultivation system still contains nutrients and PPPs (if applied in the cultivation) and needs to be treated to remove 95% of PPP. Prior to treatment, discharged water is stored separately and to reduce cost, the amount of discharge is kept as low as possible. According to Dutch regulations, the water needs to be treated with approved purification equipment [45]. As the infrastructure for transport of water is expensive, water treatment is best done in-house. Several options are available: dedicated equipment, combined systems also for disinfection, or even a mobile carry-in service. In concentrated greenhouse areas, multiple neighbouring horticultural enterprises could decide to treat their discharge water at a central location. This however requires a strong commitment of the growers. Also, earlier studies showed that the implementation of collective treatment of wastewater flows (including nutrients) from (semi-) closed systems appeared to be rather expensive [30]. The use of on-line sensors that can measure the specific PPP used in the greenhouse can help to reduce treatment time. Hand-held and sample systems for monitoring PPP can be used to check the performance of the water treatment units, for occasional failures or to determine common treatment times.

**Zero liquid discharge cultivation (ZLD).** The ultimate step in simultaneously solving emission problems for nutrients and PPP is to achieve zero-emission by avoiding any periodical discharge. This requires good quality irrigation water and using optimal control of the quality of the recirculating nutrient solution. To prevent unbalances in nutrients, the fertigation must be based on plant needs. The whole system requires water treatment units, filters, and sufficient storage volumes and adequate piping. A ZLD cultivation system requires even more attention to the quality of inputs and the recirculating nutrient solution, compared to a semi-closed system. The use of NPK and PPP sensors might support the effective use of such systems.

### Use of sensors

The above-described overview of horticulture in the Westland Region gives opportunities to use NPK sensors as well as sensors able to measure individual PPP. Below a short description where to use them adequately.

### *NPK sensor*

The NPK-sensor can both be used by growers and water authorities.

A **grower** can use the NPK sensor for the following goals:

- In-line measuring during cultivation in the drain and in the supply side to optimise the supply to the plants. Accurate sensors are required to be used for calculation and adaptation of the composition of the nutrient solution. Dosing equipment measures EC, but within the EC all essential elements may vary but need to be within limited margins. Now once in 1-3 weeks the composition is analysed at a laboratory.
- Hand-held measuring of NPK to control delivery by the automatic equipment. The grower may check the water in the drain tank or the supply or below the dripper to see if NPK are at the right level.
- Hand-held measuring of NPK to check for leakages in the greenhouse. Reasons for leakage might be the failure of connections, an overflow of troughs, growing of algae, clogging of troughs, staff caught with equipment on drippers. Water pools develop and measuring on the NPK solves where it comes from. Leakages in soilless cultivation is one of the causes of ongoing emissions to water bodies [46], even within a ZLD strategy.
- Hand-held measuring of NPK around his farm to check on leakages in ditches or pipes. If certain water flows appear, the question is: "Is it clean or fertilized water?" Accuracy of the equipment might be less than for instance for in-line monitoring practices; an indication of origin is sufficient here.

The **water authorities** are responsible for the quality of the surface water. For this the upholders daily visit a part of their work area in the Westland Region (Figure 31). For this a group of so-called maintainers visit canals and ditches and sample them. They also have fixed measuring locations (Figure 37). The approach of the last few years is to investigate the water quality within a hydrological unit (*e.g.,* a polder) by visiting the growers and talk to them instead of direct fining them. They hope for a better behaviour by an increased commitment. Together they look to various discharge and leaking points within and around the greenhouse. For them a hand-held NPK meter can be used for:

- Frequent measuring to get an indication of the presence of the nutrients. Accuracy might be less (5-10 mg/l $NO_3$). Normal is below 2 mg/l, above 10 mg/l rapid action is required. It may lead to direct discharge of unwished nutrients in the surface water.
- Measuring pipe outlets: "Is it pure water flowing out or are there higher values of NPK measurable?" Illegal discharge might be detected earlier and juridically easier proven.
- Measuring at fixed places: to get a good impression of the water quality variation during the year; each 2-4 weeks the same place is measured resulting in a time series of data indicating the variability in quality of the surface water.

Recently, experience has been gained in a test polder with measuring nitrate using colour strips and a smartphone, but this method, although cheap, is not practical for growers and is not sufficiently reliable. A robust and portable measuring device that can measure nitrate in surface water and process water electronically, instantaneously could be very helpful for the water authorities.

***Figure 38. Traditional pipes leaching to the ditch (left). Sampling by Delfland Water Authority (right top). Fixed measuring point in a ditch to measure NPK (right bottom).***

### *Plant Protection Products biosensor*

It is expected that the biosensor for measuring individual PPP will be used by the water authorities. The concentrations in the drain water of the growers are that low (0-2 mg/l active ingredient) that mostly no effect might be expected against plagues and diseases. Those concentrations are also not harmful to the plants, so a grower is not very interested in measuring.

However, those concentrations are high in surface water and may influence biodiversity in the aquatic environment. All PPP for soilless cultivation are only approved if their concentration in the surface water is below a certain norm or threshold level. For soilless cultivation, the Greenhouse Emission Model (GEM) is in operation in The Netherlands, but not yet in Europe [41, 47]. For soil-bound crops it is still under development and comes in 2021. The model describes the water uptake by a crop and, because of the sodium concentration of the irrigation water, the required discharge in combination with filter cleaning. This output is used as input for a substance model in which a PPP is applied at a certain date and the emission concentration can be calculated. Next is that the concentration of the substance comes into a standardised ditch to determine the environmental effect.

As a biosensor can measure individual PPP, per location or per work field (*e.g.*, greenhouse horticulture, bulb growing, arable farming), specific representative sensors for certain PPP should be developed. Now imidacloprid and pirimicarb were chosen (D3.1) after an inquiry among the partners, but it can already be seen that the use of imidacloprid is decreasing and that in a couple of years another representative PPP must be chosen and developed as biosensor. A next step would be that approval of a new PPP is only possible if a biosensor is available.

As a fixed measuring spot in the surface water, as shown in Figure 37, a biosensor can also be placed there as a continuous observation of the quality of the surface water. Probably more than one sensor is required. Wouldn't it be interesting to have all dots in Figure 37 measured by a biosensor?

# 6. Literature

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