

## Article

# Identification of Mint scents using a QCM based E-Nose

Salih Okur <sup>1,2,\*</sup>, Mohammed Sarheed <sup>3</sup>, Robert Huber <sup>2</sup>, Zejun Zhang <sup>1</sup>, Lars Heinke <sup>2</sup>, Adnan Kanbar <sup>3</sup>, Christof Wöll <sup>1</sup>, Peter Nick <sup>3</sup>, Uli Lemmer <sup>2,4</sup>

<sup>1</sup> Institute of Functional Interfaces (IFG), Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344, Eggenstein-Leopoldshafen, Germany; [salih.okur2@kit.edu](mailto:salih.okur2@kit.edu), [lars.heinke@kit.edu](mailto:lars.heinke@kit.edu), [christof.woell@kit.edu](mailto:christof.woell@kit.edu)

<sup>2</sup> Light Technology Institute, Karlsruhe Institute of Technology, Engesserstraße 13, 76131 Karlsruhe, Germany; [salih.okur2@kit.edu](mailto:salih.okur2@kit.edu), [robert.huber@kit.edu](mailto:robert.huber@kit.edu), [uli.lemmer@kit.edu](mailto:uli.lemmer@kit.edu)

<sup>3</sup> Karlsruhe Institute of Technology, Botanical Institute, Molecular Cell Biology, Fritz-Haber-Weg, 76131 Karlsruhe, Germany; [mohammed.sarheed@kit.edu](mailto:mohammed.sarheed@kit.edu), [adnan.kanbar@kit.edu](mailto:adnan.kanbar@kit.edu), [peter.nick@kit.edu](mailto:peter.nick@kit.edu)

<sup>4</sup> Institute of Microstructure Technology, 223, Karlsruhe Institute of Technology, Hermann-von-Helmholtz-Platz 1, Eggenstein-Leopoldshafen, Karlsruhe, 76344, Germany; [uli.lemmer@kit.edu](mailto:uli.lemmer@kit.edu)

\* Correspondence: [salih.okur2@kit.edu](mailto:salih.okur2@kit.edu); Tel.: (+49-721-608-26078)

**Abstract:** Mints emit diverse scents that exert specific biological functions and are relevance for applications. The current work strives to develop electronic noses that can electronically discriminate the scents emitted by different species of Mint as alternative to conventional profiling by gas chromatography. Here, 12 different sensing materials including 4 different metal oxide nanoparticle dispersions (AZO, ZnO, SnO<sub>2</sub>, ITO), one Metal Organic Frame as Cu(BPDC), and 7 different polymer films including PVA, PEDOT:PSS, PFO, SB, SW, SG, PB were used for functionalizing of QCM sensors. The purpose was to discriminate six economically relevant Mint species (*Mentha x piperita*, *Mentha spicata*, *Mentha spicata* ssp. *crispa*, *Mentha longifolia*, *Agastache rugosa*, and *Nepeta cataria*). The adsorption and desorption datasets obtained from each modified QCM sensor were processed by three different classification models including Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA), and k-Nearest Neighbor Analysis (k-NN). This allowed discriminating the different Mints with classification accuracies of 97.2% (PCA), 100% (LDA), and 99.9% (k-NN), respectively. Prediction accuracies with a repeating test measurement reached up to 90.6% for LDA, and 85.6% for k-NN. These data demonstrate that this electronic nose can discriminate different Mint scents in a reliable and efficient manner.

**Keywords:** Mint, Plant volatiles, Electronic Nose, Principal Component Analysis, Linear Discriminant Analysis, k-Nearest-Neighbors Analysis.

## 1. Introduction

As sessile organisms, plants have to rely on chemistry to cope with their biotic environment. As a result, they have evolved an elaborate and proficient secondary metabolism. So far, an estimated 100 000 of compounds specific for plants have already been identified [1]. These compounds include volatile compounds that are signals with the function to steer the interaction of neighboring plants or the interaction with other organisms, such as insects or microorganisms. For instance, in response to an attack by caterpillars, tomato plants warn their neighbors by emitting (Z)-3-hexenol, such that these neighbors can already synthesize defense compounds prior to being attacked [2]. A specific subset of plant volatiles act to inhibit growth or development of their competitors, a phenomenon termed as allelopathy [3, 4].

The profile of such volatile compounds can differ even between closely related species of the same genus, indicating a high degree of specificity. A classic example are the

Mints (*Mentha* spec. and neighboring genera of the *Mentheae*), where each species is endowed with a characteristic and specific bouquet of volatile compounds that are emitted from glandular hairs [5]. This specificity may relate to the fact that the sending plants needs to evade self-inhibition. A given species of Mint does not respond to this signal by itself, while its neighbors are under extreme stress. In case of a signaling compound, one way to evade self-inhibition would be to modify the respective receptor in a way that the ligand cannot bind anymore. However, there are other possible mechanisms, such as sequestered release, or metabolic conversion of the compound. Irrespective of this aspect, the allelopathic effect of Mint scent is well known. For instance, Peppermint (*M. x piperita*) can block the germination of Mediterranean weeds [6, 7]. The effects are often species specific. For instance, a comparative analysis of germination inhibition in combination with activity-guided fractionation revealed that menthone / isomenthone released by Korean Mint (*Agastache rugosa*) caused a swift and complete breakdown of microtubules in the target plant such that germination (requiring microtubules for cell division and cell expansion) is blocked, while closely related compounds such as menthol, were ineffective[8]. This specificity of biological action may be valorized for the development of novel, environmentally compatible bioherbicides that specifically affect a certain type of weed without damaging the useful crop [9]. The biological specificity of Mint oil is also of commercial relevance. Peppermint (*M. x piperita*), Spearmint (*M. spicata*), and Corn Mint (*M. canadensis*) differ in their oil composition. For instance, Spearmint is rich in carvone, making it interesting as spice [10], while Corn Mint is commercially relevant as the richest source of natural menthol[11]. The authentication of Mint oil is therefore subject to legal regulation, but, so far, has to rely on time consuming and skill-requiring technology. For instance, to discriminate oil from *M. x. piperita* and *M. arvensis*, the Japanese Custom Authorities have established a sophisticated Gas Chromatography GC-MS method [12]. The availability of user-friendly, specific, and easy-to-handle alternatives would safeguard a lot of time, labor, and costs.

A practical and intelligent sensing technique, such as QCM type electronic noses can be used as a powerful method to electronically discriminate plant volatiles, which is of relevance for applications in the plant sciences, agriculture, forestry [13], and plant biotechnology [14-16]. Electronic noses with an array of chemical sensors have been successfully utilized to discriminate volatile odorant components emitted from medical plants such as peppermint versus spearmint [17]. The diversity of the chemical sensor materials such as metal oxides [18, 19], semiconductors[20], nanoparticles and nanowires[21], polymers and small organic molecules with functional sites [22] increase the discrimination capability of e-nose sensor arrays and their applications [23, 24], since each different sensing element measures a different property of the sensed chemical materials and contributes as an extra fingerprint. Recently, a sensor array made of Surface-Anchored Metal-Organic Frameworks (SURMOFs) was successful in the detection and discrimination of plant oil scents and their mixtures. [25, 26]

In the current work, 12 different sensing materials including metal oxide nanoparticle dispersions, SURMOFs and polymer thin films for the modification of QCM sensors allowed to discriminate six different Mint species. These included Pepper Mint, *M. x piperita* (PM), Horse Mint *M. longifolia* (MLF), Korean Mint *Agastache rugosa* (AR), Cat Mint *Nepeta cataria* (NCL), and even two chemotypes of the same species of Spearmint *Mentha spicata* var. *spicata* (MS), and *Mentha spicata* var. *crispa* (MC). The adsorption and desorption datasets obtained from each sensor were used for the discrimination and prediction of those scents using three different classification methods i.e. PCA, LDA, and k-NN techniques. We show that this set-up allows for differentiation between essential oils from these species with high accuracy and efficiency, even down to the level of chemotypes belonging to the same species. Our work paves the way for new and versatile applications in quality control and product authentication as alternative to the currently used GC-MS based approaches.

2. Materials and Methods

2.1. Plant material and extraction of essential oils

The study included six species of Mints grown in the Botanical Institute of Karlsruhe Institute of Technology (KIT), Germany (Table 1). Since Mints are chemically diverse and taxonomically demanding, all accessions had been authenticated by morphological and molecular markers [27]. The scents were collected from 1 g fresh weight of leaves. Due to the different leaf size, this comprised different numbers of leaves. The data set included members of the genus *Mentha*, as well as from two neighboring genera of the *Mentheae* (*Agastache*, *Nepeta*). In addition, for one species (*M. spicata*), two different chemotypes were tested, whether the approach provided resolution beyond the species level.

**Table 1:** Accessions used in this study. The voucher number gives the code, under which the plants are available in the Botanical Garden of the KIT. The number of leaves harvested to reach 1 g is indicated as well.

Mint accession	Common name	Abbreviation	KIT voucher	Number of leaves for 1 g
<i>Mentha x piperita</i> L.	Pepper	PM	5393	10
	Mint			
<i>Mentha longifolia</i> (L.)	Horse Mint	MLF	8682	10
<i>Mentha spicata</i> L. var. <i>spicata</i>	Spear Mint	MS	7579	23
<i>Mentha spicata</i> L. var. <i>crispa</i> (MC)	Curly Mint	MC	5391	7
<i>Agastache rugosa</i> (Fisch. & C.A.Mey)	Korean	AR	7576	12
Kuntze	Mint			
<i>Nepeta cataria</i> L.	Catnip	NCL	4643	11

2.2. Chemicals and fabrication of sensors

Table 1 summarizes the commercially obtained nanoparticles with various size, dispersions used in this work. ZnO nanoparticles with a size of 10-15 nm with (2.5% w/v. in isopropanol (Avantama AG) were diluted to 1.25%. Al doped ZnO nanoparticles N-21X-Jet (AZO) (Avantama AG) with 2.5% w/v in ethanol were diluted to 1.25% w/v. SnO<sub>2</sub> nanoparticles 2.5% w/v. in butanol (Avantama AG) were diluted to 1.25% w/v. ITO nanoparticles with the size of less than 100 nm, and with 30% w/v in isopropanol (Sigma-Aldrich) were diluted to 0.6% w/v. Spiro-based conjugated polymers such as SPW-111 (SW), SPG-01T (SG), SPB-02T (PB) from Merck KGaA, and the conjugated polymer ADS229BE (PFO) from American Dye Source were selected as sensing thin film material for the QCM array. The sensor polymers were dissolved in toluene to a concentration of 5 g/l. The photophysical properties have been published previously [28, 29]. Poly(vinyl alcohol) 99% hydrolyzed (PVA) purchased from Aldrich was dissolved in distilled water. Poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS) 2.8 % w/v dispersion in H<sub>2</sub>O was purchased from Merck. The Cu(BPDC) MOF thin-film (Copper Biphenyl-4,4'-Di-Carboxylic Acid) structures were prepared in a layer-by-layer fashion, following an optimized synthesis protocol published previously [30]. Alternatively, the samples were prepared by alternately exposing the substrate to the metal node and to the linker solutions, using a spray method [31, 32]. A MOF thin Cu(BPDC) film [33] was prepared from ethanolic 1 mM copper acetate and ethanolic 0.2 mM biphenyl dicarboxylic acid (BPDC). Prior to SURMOF synthesis, the QCM substrates were functionalized by plasma treatment for 30 min. Cu(BPDC) was prepared in 30 synthesis cycles.

Commercially available HC-49U type silver coated 10 MHz quartz crystals produced as an electronic element (JWT, China) were used as QCM electrodes. Before spin coating, the QCM sensors were sonicated in acetone and isopropanol for 10 minutes sequentially. After drying on a hot plate at 80°C for 5 min, 10 µL of dispersed nanoparticles were used for spin coating with a spin speed between 1000-2000 rpm with 30s spinning time. In case of the polymers, all samples were prepared with a solution of 5 g/l for spin coating, and a

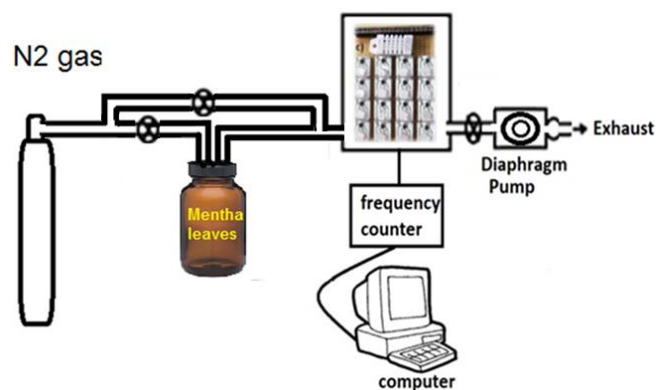
spin speed between 1300-2100 rpm with 30 s spinning time. Following the coating, the samples were annealed at 80°C for 10 min on a heating plate. Table 2 shows the frequency shifts after spin coating that ranged between 7-82 kHz, depending on the material.

**Table 2.** Frequency shifts after spin coating.

Materials	Spin Speed (RPM)	Initial f <sub>0</sub> (MHz)	Frequency Shift (kHz)
ZnO	2000	9,9998	45.1
AZO	2000	10,0000	43.6
SnO <sub>2</sub>	1000	9,9999	36.5
ITO	1000	9,9996	81.8
PVA	2000	10,0200	51.8
PEDOT.PSS	2000	10,0001	50.4
PB	2100	10, 0064	7.5
SW	1550	10, 0154	9.2
PFO	1300	10,0061	9.7
SB	2100	10,0021	16.7
SG	1300	10,0061	8.4
Cu(BPDC)	-	10,0001	13.9

### 2.3. Data acquisition with the e-nose

Figure 1 shows the working principle of the 12-channel homemade E-nose system used for the Mint scent adsorption/desorption process and data collection. A 400-ml stainless cylindrical chamber was used for the sensor array. The chamber was first evacuated for 10 min with a diaphragm pump and then purged with dry N<sub>2</sub> gas for 10 min with a flow rate of 10 L.min<sup>-1</sup> before each measurement. For each Mint accession, 1 g fresh weight of freshly collected leaves was placed separately into a 100 ml glass bottles for the measurement. The emitted scents from the leaves inside the bottles were transferred to the chamber with 10 mL.min<sup>-1</sup> of N<sub>2</sub> flowing into the chamber with the sensor array. For each Mint accession, the change in resonance frequency was followed with 2 cycles of adsorption over 30 min exposure, and subsequently, 60 min desorption during cleaning with dry N<sub>2</sub> gas.



**Figure 1:** E-nose setup used for measuring the Mint scent.

### 2.4. Data Analysis and Classification



The first cycle of the adsorption/desorption curve was used for the discrimination of the scents, while the second cycle was used for testing and prediction or identification of the six different scents emitted from the plant source under investigation. For this purpose, intervals of the frequency curves extending over 10 min were excised from the final range of the adsorption phase (20-30 min after the onset of adsorption, when responses were maximal) and used for the training for the discrimination of the six different Mint samples. Three different classification algorithms were tested: PCA, LDA, and k-NN based on scripts written in MATLAB. A cross-validation threshold of 10-fold was used for classification, using 1800 individual observations. Hereby, 1620 observations were collected during the first cycle and represented the training set, and 180 observations were chosen randomly from the second cycle and represented the identification set. The data sets for training and for identification data sets were obtained by repeating the experiment under the same conditions. For the identification (second cycle), similarly, a time interval of 10 min from the adsorption phase was recorded, when the response was maximal (between 110-120 min from the start of the experiment). This frequency course served for the identification of the specimen.

For the QCM measurements, Sauerbrey's equation [34] was used to verify the linear dependence of the QCM frequency shift on the mass upload on the piezo electric sensor [25].

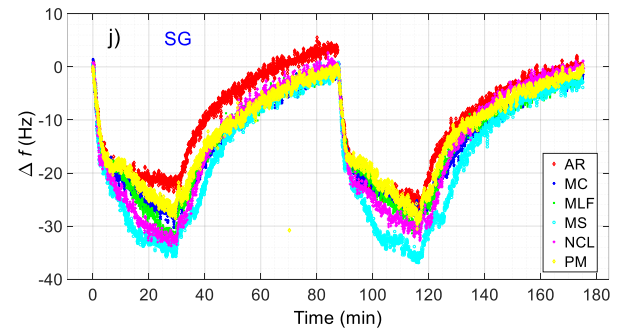
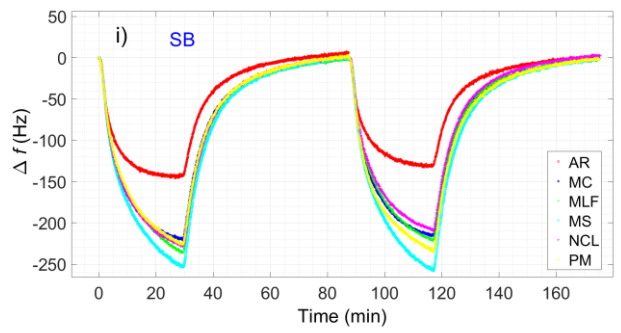
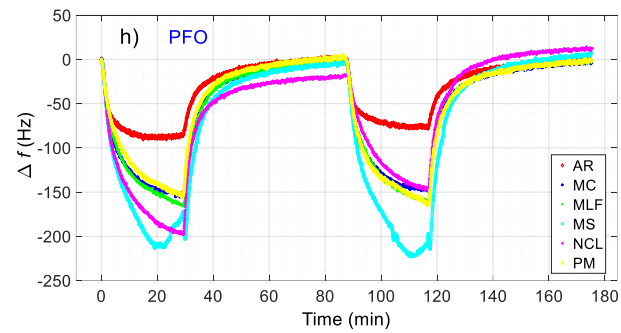
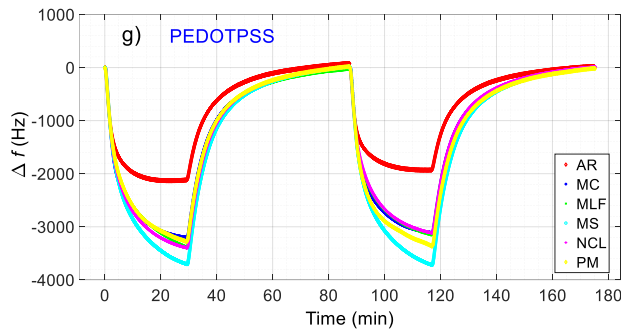
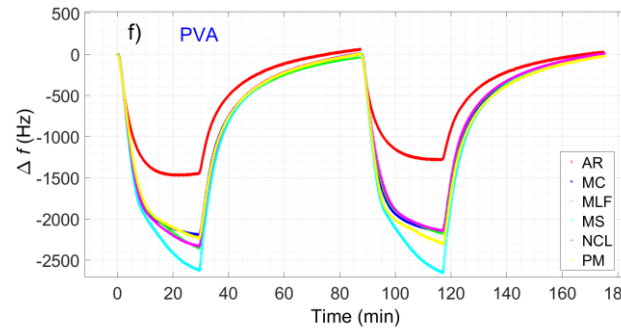
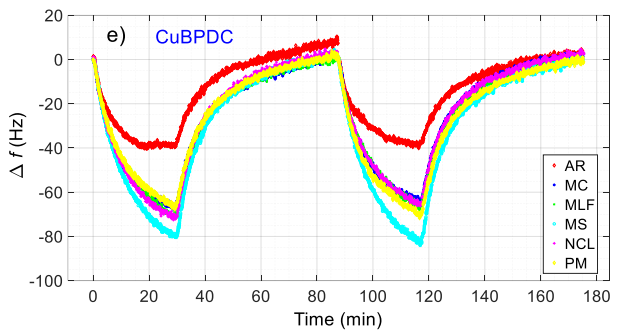
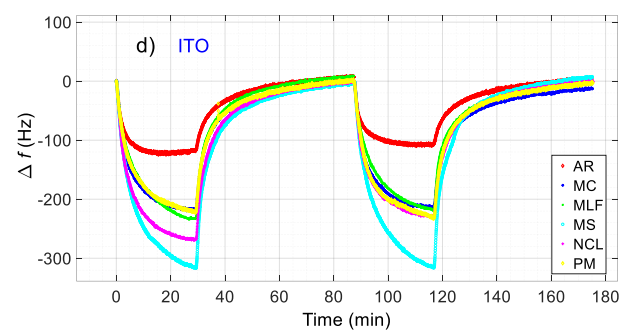
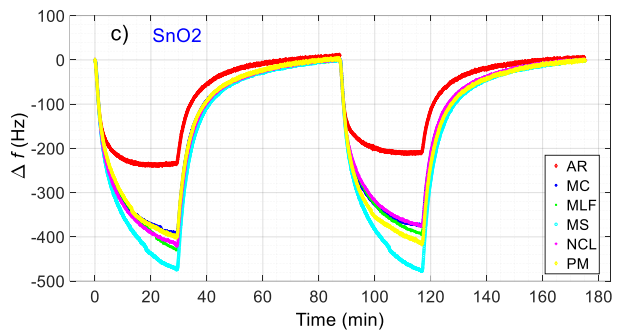
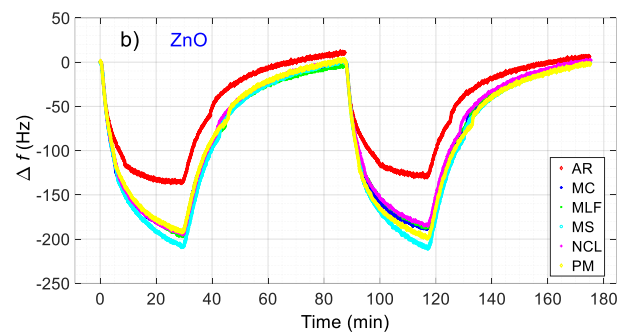
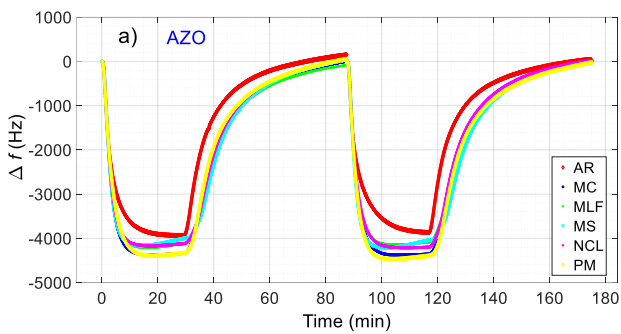
PCA was used as one the most effective quantitative methods to discriminate volatile gasses and odors [35-37]. In PCA, a new set of variables derives as principal components from a linear combination of the original variables, being orthogonal to each other. The criterion to define this pair of variables, is that their variance is maximal among all the other possible choices of the first axis. LDA is closely related to PCA and explicitly attempts to maximize the variance difference between data classes while minimizing the variance differences inside each individual class. Hereby, the fitting function estimates the parameters assuming linear boundaries between classes.

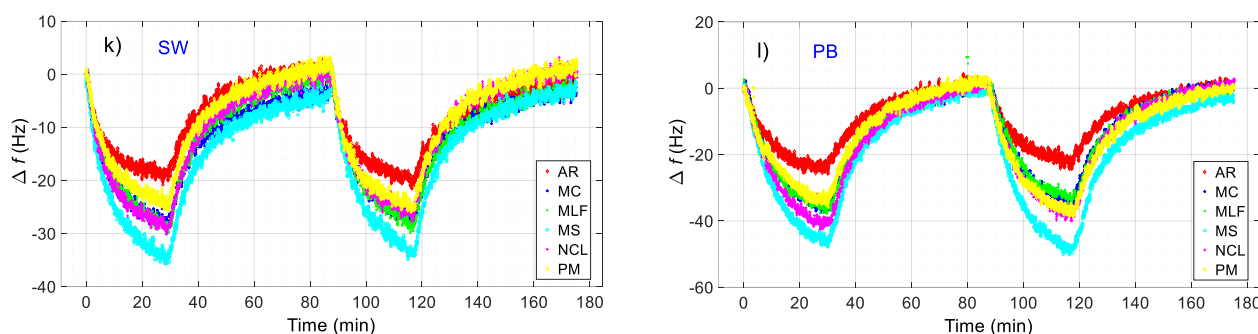
In the k-Nearest Neighbor classification, the algorithm searches and classifies an unknown point by counting each number of closest members of already known neighboring classes. Each known object contributes for its class, and the closest class with the highest number of members yields the predictive decision. In this work, for the k-NN discrimination, k was chosen as 10, and the effect of the number of up to 300 neighbors on the discrimination accuracy of k-NN model was estimated based on Euclidean distances.

### 3. Results and Discussion

#### 3.1. Sensor array responses

Figure 2 illustrates the changes in resonance frequencies of the sensor array with 12 different sensing materials as 2 cycles of adsorption (30 min) after exposure to each fresh Mint leaf and 60 min desorption by cleaning with dry N<sub>2</sub> gas. In general, each sensor showed different cyclic response to each emitted concentration of each Mint scent. AZO produced the highest response among the sensor array and saturated within a few minutes, while most of the remaining sensors reached saturation only upon exposure to *Agastache rugosa* (AR) scent with the lowest response.

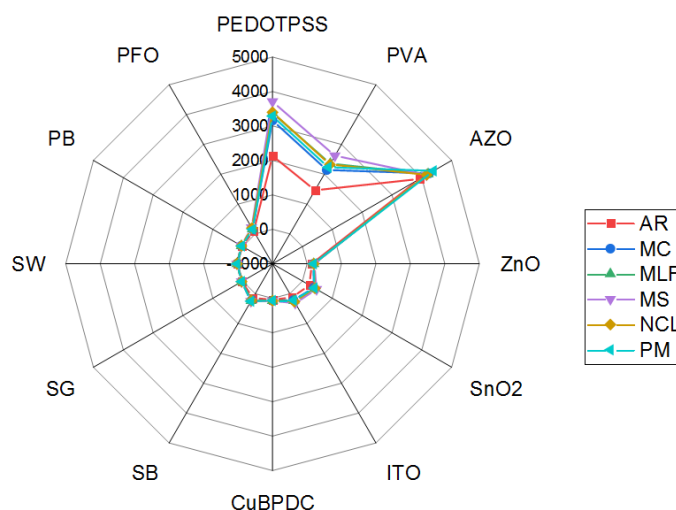




**Figure 2.** Resonance frequency shifts of the sensor array with 12 different sensing materials (see Table 2 for abbreviations) during two cycles of exposure to the individual Mint leaves (see Table 1 for abbreviations).

A radar plot of the maximum frequency shifts responses for the sensor arrays is given in Figure 3 for comparison. This radar plot, too, shows a clear discrimination. All the sensors exhibit a maximal response upon exposure to the scent of *Mentha spicata* (MS), and the lowest response upon exposure to the scent of *Agastache rugosa* (AR). On the other hand, the responses to the scents of the remaining Mints (i.e. MC, PM, MLF and NCL) are very close to each other.

AZO produces the highest response with -4450 Hz as change in the resonance frequency, while the sensor with ZnO nanoparticles exhibits the lowest response among all sensors with only -220 Hz. Likewise, PEDOT:PSS displays a high response with -3722 Hz, while the sensor with SW and SG yield the lowest response among the polymer sensors with around -35 Hz. Also, the Cu(BPDC) as SURMOFs are not very responsive with around -80 Hz as change in the resonance frequency.



**Figure 3.** Maximum frequency shifts responses of sensor array.

### 3.2. Principal Component Analysis (PCA)

Figure 4 shows a 2D plot for the coefficients of the Principal Component Analysis from 1800 observations for the six different Mint scents that group into six different clusters. Each scent is shown in different colors for visual discrimination. While these clusters separated from each other, the borders between Pepper Mint (PM) and Curly Mint (MC), as well as between Horse Mint (MLF) and Cat Mint (NCL) are not well defined. The sum of the 2 percentages of the total variance explained by each principal component is equal to 97.2. This show very high discrimination accuracy close to 99% as compared to the rest of the variance components.

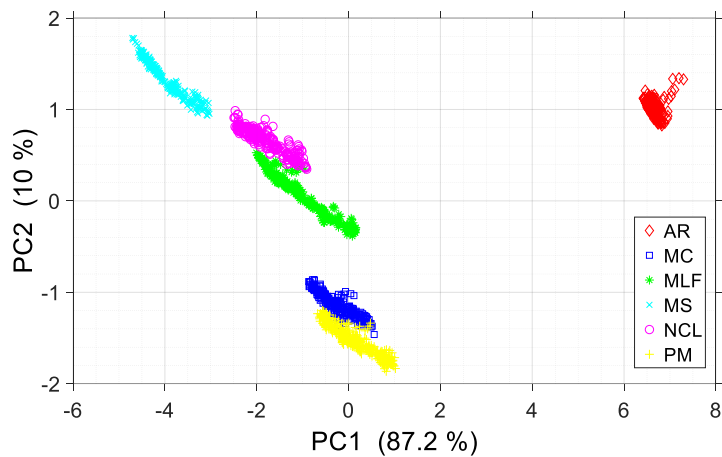
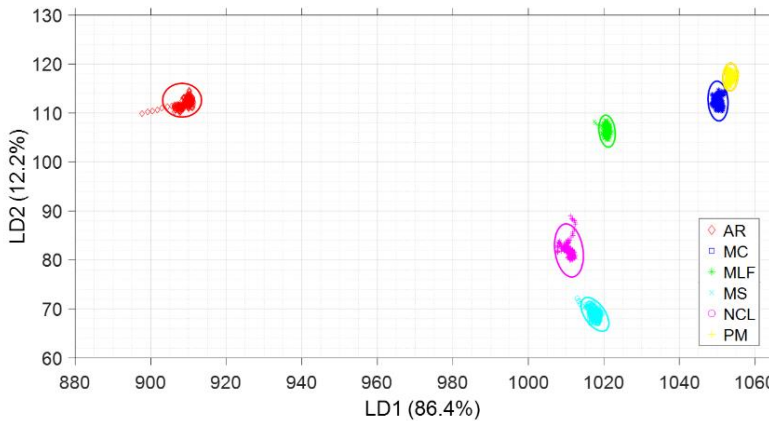


Figure 4: 2D plot of the Principal Component coefficients from 1800 observations.

3.3. Linear Discrimination Analysis (LDA)

Figure 5 shows a 2D plot of the Linear Discriminant Analysis with the 95% confidence ellipses obtained for the different species (Fig 5a) obtained from 10 rounds of LDA calculation using the data obtained from the first cycle of the e-nose measurements given in Fig 2. The LDA plot shows a clear visual discrimination for the six types of Mints. The sum of the first two components of the LDA vector components is 98.6%, and the discrimination accuracy with the LDA method reached 100%, even though the LDA scores obtained for *Menta spicata crispa* (MC, blue) and *Mentha x piperita* (PM, yellow) are very close to each other.

To get a further readout for the quality of the prediction, a so-called confusion matrix was calculated (Fig 5b). A confusion matrix is a chart for comparison of the predicted (identified) labels with the true labels. The rows of the confusion matrix correspond to the true class and the columns correspond to the predicted class. Diagonal and off-diagonal cells correspond to correctly and incorrectly classified observations, respectively. The obtained confusion matrix also confirms the discrimination accuracy without any misclassification between groups of ten training data sets and randomly chosen test data sets. In this figure, the first two diagonal cells show the number and percentage of correct classifications by the trained observation data sets. For instance, in 291 cases scents from *Agastache rugosa* (AR) scents were correctly classified corresponding to 16% of all 1800 observed data sets. Since there was no single case of misclassification, the discrimination value was 100% correct.



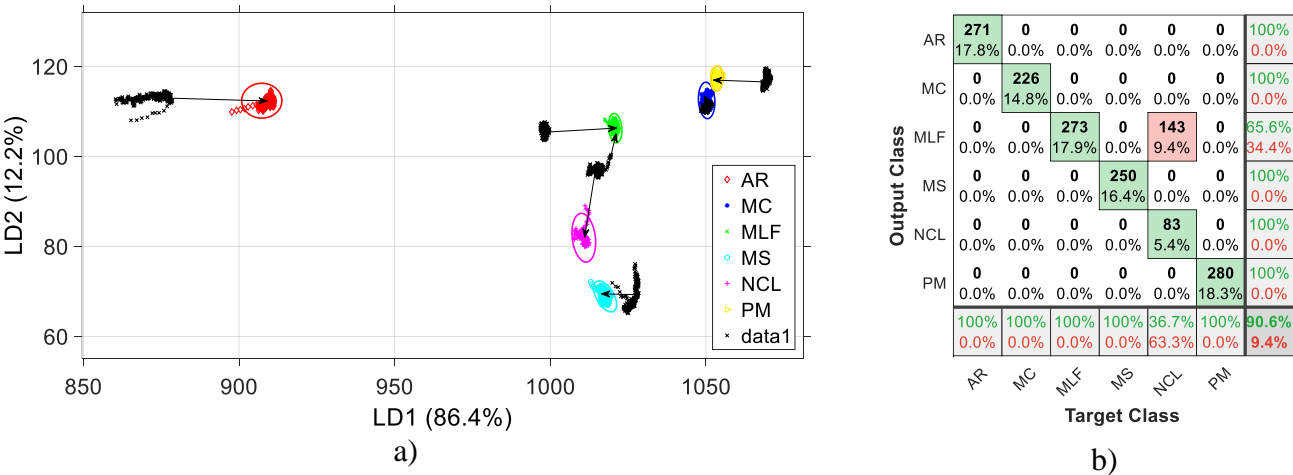
a)

Output Class	AR	291 16.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	100% 0.0%
	MC	0 0.0%	271 14.9%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	100% 0.0%
	MLF	0 0.0%	0 0.0%	330 18.1%	0 0.0%	0 0.0%	0 0.0%	100% 0.0%
	MS	0 0.0%	0 0.0%	0 0.0%	300 16.5%	0 0.0%	0 0.0%	100% 0.0%
	NCL	0 0.0%	0 0.0%	0 0.0%	0 0.0%	262 14.4%	0 0.0%	100% 0.0%
	PM	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	366 20.1%	100% 0.0%
		100% 0.0%	100% 0.0%	100% 0.0%	100% 0.0%	100% 0.0%	100% 0.0%	100% 0.0%
		Target Class						
		AR	MC	MLF	MS	NCL	PM	

b)

**Figure 5.** Linear Discriminant Analysis of six species of Mints. (a) 2D plot of the Linear Discriminant Analysis with 95% confidence ellipse, (b) confusion matrix obtained from 10-fold LDA calculations using the data obtained from the first cycle of the e-nose measurements.

Figure 6a shows a 2D plot of the 10-fold Linear Discriminant Analysis obtained from the training data sets. The colored symbols represent the measurements from the first cycle, while the black symbols give the data from the second cycle of the e-nose measurements. The LDA vector points calculated from the data sets from second cycle of the e-nose measurement allowed prediction of the unknown observations. The six different Mints segregate into fully distinct clusters. The first two components of the LDA vector components sum up to more than 99% reflecting the good separation of these clusters. With exception of Cat Mint (NCL), prediction classes (obtained from the second cycle) localized closely to the training classes (obtained from the first cycle) in the 2D LDA plot. For Cat Mint, the prediction was located just between Cat Mint (NCL, purple symbols) and Horse Mint (MLF, green symbols). This caused a 63.3% misclassification for the Cat Mint data sets in the corresponding prediction-confusion matrix derived from the unknown data sets collected during the second cycle of the e-nose measurements (Figure 6b). However, the overall prediction accuracy for the unknown data sets was very high with 90.6% meaning that only 9.4% of the data remained mis-classified.



**Figure 6.** Linear Discriminant Analysis of six Mint species. (a) 2D plot of the 10-fold Linear-Discriminant Analysis obtained from the training data sets shown with the colored symbols (first cycle e-nose measurements), and from the prediction data sets shown with the black symbols (second cycle of the e-nose measurements), (b) prediction confusion matrix with the unknown data sets from the second cycle of the e-nose measurements.

3.4. Nearest Neighbor Analysis (k-NN)

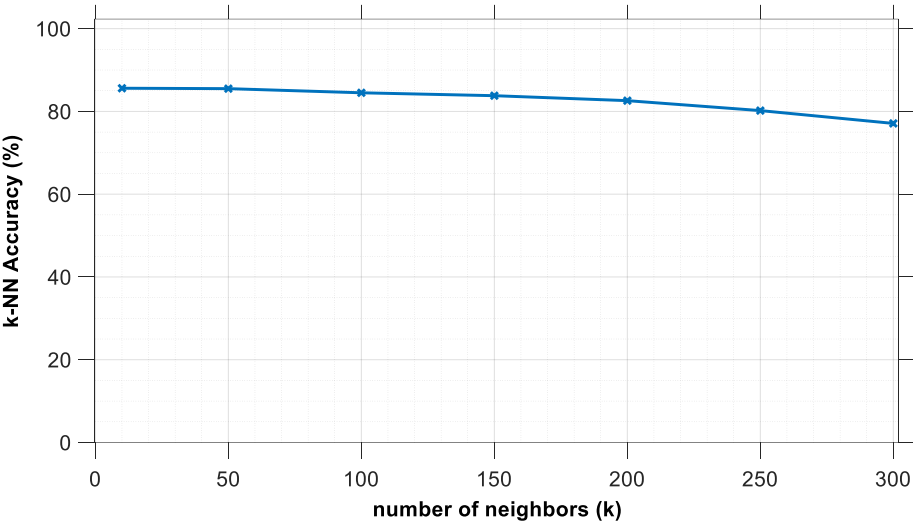
As last algorithm for prediction, we used k-NN analysis with a 10-fold (k=10) calculation of the unknown data sets from the second cycle as compared to the true assignment from the training data set collected during the first cycle. The data sets from the second



cycle of the e-nose measurement was used for the k-NN calculation to determine prediction accuracy for the unknown observations. Again, Cat Mint (NCL) and Horse Mint (MLF) remained ambiguous with 94.7% misclassification. The overall prediction accuracy for the unknown data sets was poorer than in case of LDA with 85.6% corresponding to 14.4% misclassification.

Output Class	AR	MC	MLF	MS	NCL	PM	
	271 17.8%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	100% 0.0%
	0 0.0%	220 14.4%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	100% 0.0%
	0 0.0%	0 0.0%	273 17.9%	0 0.0%	192 12.6%	0 0.0%	58.7% 41.3%
	0 0.0%	0 0.0%	0 0.0%	250 16.4%	0 0.0%	0 0.0%	100% 0.0%
	0 0.0%	0 0.0%	0 0.0%	0 0.0%	12 0.8%	0 0.0%	100% 0.0%
	0 0.0%	6 0.4%	0 0.0%	0 0.0%	22 1.4%	280 18.3%	90.9% 9.1%
	100% 0.0%	97.3% 2.7%	100% 0.0%	100% 0.0%	5.3% 94.7%	100% 0.0%	85.6% 14.4%
	AR	MC	MLF	MS	NCL	PM	

**Figure 7.** The prediction confusion matrix obtained from the k-NN analysis with10-fold (k=10) calculation with the unknown data sets from the second cycle to compare with the training data set (true labels) obtained from the first cycle.



**Figure 8.** k-NN accuracy (%) with increasing number of nearest neighbor

In the next step, we tested to what extent the accuracy of the k-NN algorithm was dependent on the number of neighbors (Fig. 8). However, while increasing k from 10 up to 300, we did not see any further increase in accuracy. In contrast, the accuracy dropped slowly. Thus, the distances between the classes sufficiently separate when using 10 neighbors.

4. Conclusions

Twelve different sensing materials including four different metal oxide nanoparticle dispersions, one SURMOF, and seven different polymer films have been used for the modification of silver coated QCM sensors to discriminate six different Mint species. The adsorption and desorption datasets obtained from each modified QCM sensor have been used for three different classification models (PCA, LDA, k-NN). Classification accuracies for the training sets reached 97.2% for PCA, 100% for LDA, and 99.9% for the k-NN method (using k=10 nearest neighboring points). Prediction accuracies for a repeating test measurement with unknown samples, reached 90.6% for LDA and 85.6% for the k-NN method. The results reveal that a QCM type e-nose represents a user-friendly and reliable alternative to GC-MS to monitor and discriminate the emitted scents from different Mint species and even, beyond the species level, for different chemotypes of individual species. This paves the way for numerous applications from pharmaceutical quality control to monitoring Mint oils as environmentally friendly bioherbicides.

**Author Contributions:** “Conceptualization, S.O., M.S., A.K, P.N., and U.L.; methodology, S.O., M.S., A.K., P.N., and U.L. ; software, S.O., R.H.; validation, S.O., M.S., R.H, A.K., P.N., and U.L.; formal analysis, S.O., R.H., A.K., L.H., P.N., and U.L. ; investigation, S.O.; resources, S.O., C.W., and P.N., and U.L.; data curation, S.O., and L.H.; writing—original draft preparation, S.O., M.S., R.H., P.N.; writing—review and editing, R.H., A.K., L.H., C.W., P.N., and U.L. ; visualization, S.O. ; supervision, C.W., P.N., and U.L. ; project administration, S.O., C.W., P.N., and U.L. ; funding acquisition, C.W., P.N., and U.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy—2082/1—390761711, Philipp Schwartz Fellowship (a program run by the Alexander von Humboldt Foundation) provided by the Karlsruhe Institute of Technology, and Iraqi Ministry of Education and Science.

**Institutional Review Board Statement:** “Not applicable” for studies not involving humans or animals.

**Acknowledgments:** The corresponding authors as well as co-authors A. K. and S. O. obtained support through a Philipp Schwartz Fellowship (a program run by the Alexander von Humboldt Foundation) provided by the Karlsruhe Institute of Technology. Co-author M. S. was supported by a PhD fellowship from the Iraqi Ministry of Education and Science; U.L. and C.W. acknowledge support from Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy—2082/1—390761711. The authors would like to thank Mr. Joachim Daumann for competent cultivation and maintenance of the plant material used in this study. The authors would like also to thank Mr. Felix Geislhoeringer for preparing the circuit diagram for the 16-channel e-nose system.

**Conflicts of Interest:** No conflicts of interest.

## References

- [1] A. Goossens, S.T. Häkkinen, I. Laakso, T. Seppänen-Laakso, S. Biondi, V. De Sutter, F. Lammertyn, A.M. Nuutila, H. Söderlund, M. Zabeau, A functional genomics approach toward the understanding of secondary metabolism in plant cells, *Proceedings of the National Academy of Sciences*, 100 (2003) 8595-8600.
- [2] K. Sugimoto, K. Matsui, Y. Iijima, Y. Akakabe, S. Muramoto, R. Ozawa, M. Uefune, R. Sasaki, K.M. Alamgir, S. Akitake, Intake and transformation to a glycoside of (Z)-3-hexenol from infested neighbors reveals a mode of plant odor reception and defense, *Proceedings of the National Academy of Sciences*, 111 (2014) 7144-7149.
- [3] J.J. Ferguson, B. Rathinasabapathi, C.A. Chase, Allelopathy: How plants suppress other plants, *EDIS*, 2013 (2013).
- [4] N. Schandry, C. Becker, Allelopathic Plants: Models for Studying Plant–Interkingdom Interactions, *Trends in Plant Science*, 25 (2020) 176-185.
- [5] T.L. Weir, S.-W. Park, J.M. Vivanco, Biochemical and physiological mechanisms mediated by allelochemicals, *Current opinion in plant biology*, 7 (2004) 472-479.
- [6] A. Cavalieri, F. Caporali, Effects of essential oils of cinnamon, lavender and peppermint on germination of Mediterranean weeds, *Allelopathy J*, 25 (2010) 441-452.
- [7] E. Campiglia, R. Mancinelli, A. Cavalieri, F. Caporali, Use of essential oils of cinnamon, lavender and peppermint for weed control, *Italian Journal of Agronomy*, DOI (2007) 171-178.

- [8] M.M. Sarheed, F. Rajabi, M. Kunert, W. Boland, S. Wetters, K. Miadowitz, A. Kaźmierczak, V.P. Sahi, P. Nick, Cellular base of mint allelopathy: Menthone affects plant microtubules, *Frontiers in plant science*, 11 (2020) 1320.
- [9] E.I. Argyropoulos, D. Vokou, I. Eleftherochorinos, In vitro evaluation of essential oils from Mediterranean aromatic plants of the Lamiaceae for weed control in tomato and cotton crops, Aristotle University of Thessaloniki, 2008.
- [10] S. Kokkini, R. Karousou, T. Lanaras, Essential oils of spearmint (Carvone-rich) plants from the island of Crete (Greece), *Biochemical Systematics and Ecology*, 23 (1995) 425-430.
- [11] S. Sharma, B. Tyagi, Character correlation, path coefficient and heritability analyses of essential oil and quality components in Japanese mint, DOI (1991) 257-262.
- [12] Analysis Method of Peppermint Oil  
Japan Customs Analysis Methods 2019, pp. 3301.3324 - 3301.3325.
- [13] A.D. Wilson, Diverse applications of electronic-nose technologies in agriculture and forestry, *Sensors*, 13 (2013) 2295-2348.
- [14] S. Cui, P. Ling, H. Zhu, H.M. Keener, Plant Pest Detection Using an Artificial Nose System: A Review, *Sensors (Basel)*, 18 (2018).
- [15] J. Laothawornkitkul, J.P. Moore, J.E. Taylor, M. Possell, T.D. Gibson, C.N. Hewitt, N.D. Paul, Discrimination of plant volatile signatures by an electronic nose: aA potential technology for plant pest and disease monitoring, *Environ Sci Technol*, 42 (2008) 8433-8439.
- [16] B.-S. Noh, Analysis of volatile compounds using electronic nose and its application in food industry, *Korean Journal of Food Science and Technology*, 37 (2005) 1048-1064.
- [17] S. Kiani, S. Minaei, M. Ghasemi-Varnamkhashi, Real-time aroma monitoring of mint (*Mentha spicata* L.) leaves during the drying process using electronic nose system, *Measurement*, 124 (2018) 447-452.
- [18] X. Tian, J. Wang, S. Cui, Analysis of pork adulteration in minced mutton using electronic nose of metal oxide sensors, *Journal of Food Engineering*, 119 (2013) 744-749.
- [19] A. Berna, Metal oxide sensors for electronic noses and their application to food analysis, *Sensors*, 10 (2010) 3882-3910.
- [20] N. El Barbri, E. Llobet, N. El Bari, X. Correig, B. Bouchikhi, Electronic nose based on metal oxide semiconductor sensors as an alternative technique for the spoilage classification of red meat, *Sensors*, 8 (2008) 142-156.
- [21] M.D. Shirsat, T. Sarkar, J. Kakoullis Jr, N.V. Myung, B. Konnanath, A. Spanias, A. Mulchandani, Porphyrin-functionalized single-walled carbon nanotube chemiresistive sensor arrays for VOCs, *The Journal of Physical Chemistry C*, 116 (2012) 3845-3850.
- [22] Y. Chang, N. Tang, H. Qu, J. Liu, D. Zhang, H. Zhang, W. Pang, X. Duan, Detection of volatile organic compounds by self-assembled monolayer coated sensor array with concentration-independent fingerprints, *Sci Rep*, 6 (2016) 23970.
- [23] A.D. Wilson, M. Baietto, Applications and advances in electronic-nose technologies, *Sensors*, 9 (2009) 5099-5148.
- [24] N. Iqbal, QCM sensor arrays for monitoring volatile plant emanations via molecularly imprinted polymers, *unwiien*, 2011.
- [25] S. Okur, Z. Zhang, M. Sarheed, P. Nick, U. Lemmer, L. Heinke, Towards a MOF e-Nose: A SURMOF sensor array for detection and discrimination of plant oil scents and their mixtures, *Sensors and Actuators B: Chemical*, 306 (2020) 127502.
- [26] S. Okur, P. Qin, A. Chandresh, C. Li, Z. Zhang, U. Lemmer, L. Heinke, An enantioselective e-nose: An array of nanoporous homochiral MOF films for stereospecific sensing of chiral odors, *Angewandte Chemie International Edition*, 132 (2020) 1 - 7.
- [27] M.M. Sarheed, Allelopathic Compounds from Mint Target the Cytoskeleton from Cell Biology Towards Application as Bioherbicides, KIT-Bibliothek, 2019.
- [28] N. Bolse, R. Eckstein, M. Schend, A. Habermehl, C. Eschenbaum, G. Hernandez-Sosa, U. Lemmer, A digitally printed optoelectronic nose for the selective trace detection of nitroaromatic explosive vapours using fluorescence quenching, *Flexible and Printed Electronics*, 2 (2017) 024001.
- [29] N. Bolse, R. Huber, A. Habermehl, R. Eckstein, G. Hernandez-Sosa, A. Mertens, C. Eschenbaum, U. Lemmer, A low-cost versatile fluorescence quenching detection system for liquid-and vapor-phase sensing, 2017 IEEE SENSORS, IEEE, pp. 1-3.
- [30] O. Shekhah, H. Wang, S. Kowarik, F. Schreiber, M. Paulus, M. Tolan, C. Sternemann, F. Evers, D. Zacher, R.A. Fischer, C. Wöll, Step-by-step route for the synthesis of metal-organic frameworks, *Journal of the American Chemical Society*, 129 (2007) 15118-15119.
- [31] H.K. Arslan, O. Shekhah, J. Wohlgemuth, M. Franzreb, R.A. Fischer, C. Wöll, High-Throughput Fabrication of Uniform and Homogenous MOF Coatings, *Adv. Funct. Mater.*, 21 (2011) 4228-4231.
- [32] S. Hurre, S. Friebe, J. Wohlgemuth, C. Wöll, J. Caro, L. Heinke, Sprayable, Large-Area Metal-Organic Framework Films and Membranes of Varying Thickness, *Chemistry – A European Journal*, 23 (2017) 2294-2298.
- [33] J.X. Liu, B. Lukose, O. Shekhah, H.K. Arslan, P. Weidler, H. Gliemann, S. Bräse, S. Grosjean, A. Godt, X.L. Feng, K. Mullen, I.B. Magdau, T. Heine, C. Wöll, A novel series of isoreticular metal organic frameworks: realizing metastable structures by liquid phase epitaxy, *Sci Rep*, 2 (2012) 921.
- [34] G. Sauerbrey, G. Jung, Vibrational Modes of Planoconvex Quartz Plates, *Z Angew Physik*, 24 (1968) 100-106.
- [35] Y.-M. Yang, P.-Y. Yang, X.-R. Wang, Electronic nose based on SAWS array and its odor identification capability, *Sensors and Actuators B: Chemical*, 66 (2000) 167-170.
- [36] K. Nakamura, T. Nakamoto, T. Moriizumi, Classification and evaluation of sensing films for QCM odor sensors by steady-state sensor response measurement, *Sensors and Actuators B: Chemical*, 69 (2000) 295-301.
- [37] M. Zarzo, D.T. Stanton, Identification of Latent Variables in a Semantic Odor Profile Database Using Principal Component Analysis, *Chemical Senses*, 31 (2006) 713-724.

### Author Biography

**Salih Okur** received his diploma degree in Physics from the Hacettepe University, Turkey, in 1989 and the Ph.D. degree in Solid State Physics (May 1998) from Illinois Institute of Technology, Chicago, USA. He worked as a Research Assistant (1993–2000), an Assistant Professor (2000–2006), and Associate Professor (2006–20011) in the Department of Physics in the Izmir Institute of Technology, Turkey, before accepting a Full Professor position in the Department of Material Science and Engineering in the University of Katip Celebi. He was the Dean of Engineering Faculty in University of Katip Celebi between 2011-2015. His research area comprises nanoparticle materials, surface modification, sensors, QCM based electronic nose. Between 2017-2020, he was a Guest Professor in the Light Technology Institute (LTI) of the Karlsruhe Institute of Technology (KIT) and he is now working as Research Scientist in Institute of Functional Interfaces (IFG) at Karlsruhe Institute of Technology (KIT).

**Zejun Zhang** received his B.E. (2015) from Beijing University of Chemical Technology, in Energy Chemical Engineering, and his M.E. (2018) from the Institute of Process Engineering, Chinese Academy of Sciences. He started his PhD project at the Karlsruhe Institute of Technology in October 2018. His research concentrates on conductive, smart and stimuli-responsive MOF materials.

**Lars Heinke** studied physics in Greifswald and Leipzig and obtained his PhD in 2009. After post-doctoral stays at the Fritz-Haber Institute, Berlin, and at the Lawrence-Berkeley National Laboratory, California, he joined the Institute of Functional Interfaces at KIT as group leader. His research focus comprises of functional nanoporous films and their physical properties and photoresponsive smartcoatings.

**Christof Wöll** is Director of the Institute of Functional Interfaces at the Karlsruhe Institute of Technology (since 2009). He studied Physics at the University of Göttingen and received his PhD in 1987 at the Max-Planck-Institute of Dynamics and Self-Organization. In 1996, he took over the chair for Physical Chemistry at the University of Bochum (until 2009) and founded the collaborative research center SFB 588. He has received several awards including the van't Hoff Prize of the German Bunsen Association. He is a member of the "Deutsche Akademie der Naturforscher Leopoldina" and was the Spokesperson of the German Physical Society (DPG) Surface Physics Division (2016-2018). His research activities focus on fundamental processes in Surface Physics and Surface Chemistry, in particular development and advancement of techniques for the characterization of molecular adsorbates and oxide surfaces as well as metal-organic frameworks (MOFs and SURMOFs).

**Mohammed Sarheed** studied Agricultural Sciences in Tikrit, Iraq, before moving on to Botanical Institute of the Karlsruhe Institute of Technology (KIT) with a PhD fellowship from the Iraqi Ministry of Education and Science. He has been working on allelopathic compounds from Mint plants. He has also got a scholarship from the Karlsruhe House of Young Scientists (KHYS), a research Institute at the Federal Institute of Organic Agriculture (FiBL) in Frick.

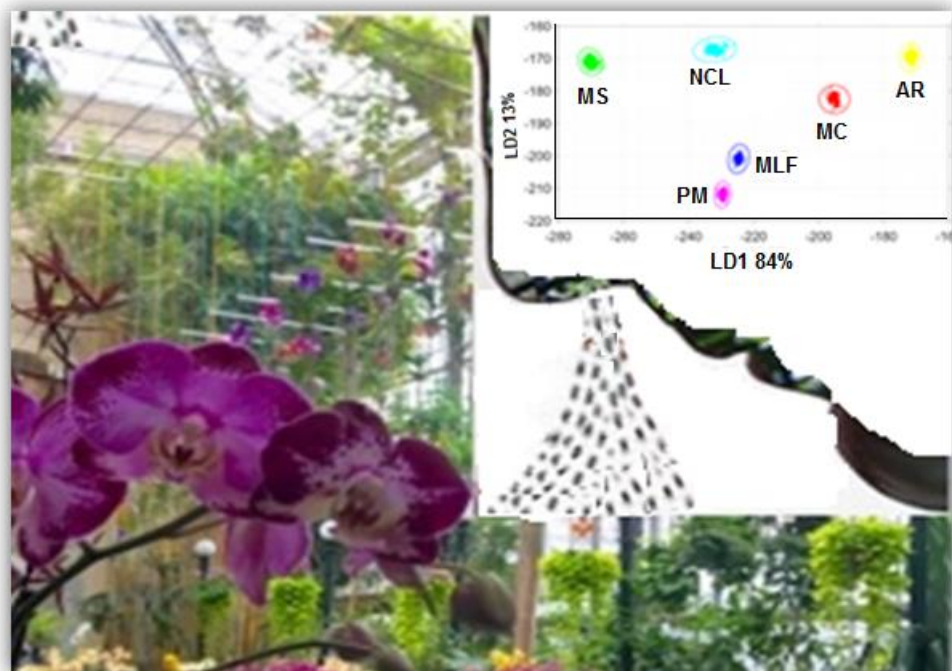
**Robert Huber** is working as a researcher and PhD candidate at Karlsruhe Institute of Technology (KIT). He achieved his master's degree in physics at KIT and proceeds his research in the electrical engineering department. His current field of research is about printed sensors. Therefore, he investigates detectors for explosives based on fluorescence quenching in microfluidic channels. In addition, he is active in Printed Electronics such as temperature sensors, which achieve a high spatial resolution as well as accurate temperature readings.

**Adnan Kanbar** Crop breeding, applied biotechnology in crop improvement (Wheat, Barley, Rice, Sorghum), Marker-assisted selection for Abiotic stresses in field crops. Developing new varieties of sweet sorghum for producing biofuel. July 2016 - Post-Doc fellowship at the Department of Molecular Cell Biology (group of Prof. Dr. Peter Nick), Botanical Institute, Karlsruhe Institute of Technology (KIT), Baden-Württemberg, Germany. March 2016 – July 2016 Post-Doc fellowship by the International Institute for Education (USA), Department of Molecular Cell Biology (group of Prof. Dr. Peter Nick), Botanical Institute, Karlsruhe Institute of Technology (KIT), Baden-Württemberg, Germany. 2010 Post-Doc fellowship under MIF (The Matsumae International Foundation, Japan), Laboratory of Plant Genetics and Breeding, Dept. of Agriculture, Faculty of Agriculture, Tokyo University of Agriculture, Japan. 2002-2006 Ph.D. scholar, Dept. of Genetics and Plant Breeding, University of Agricultural Sciences- Bangalore, India funded by a ICCR Scholarship (PhD), Government of India.



**Peter Nick** received the diploma degree in 1986 and his Ph.D. degree in 1990 in Plant Physiology from the Albert-Ludwigs University of Freiburg, Germany. After two years as research fellow at the Frontier Research Programme, Riken Institute, Saitama-ken, Japan (funded by the Japanese Science and Technology Agency), and additional two years at the Institut de Biologie Moleculaire des Plantes (CNRS) in Strasbourg France (funded by the Human Frontier Science Programme Organization), he completed his habilitation in 1996 at the Albert-Ludwig University of Freiburg (funded by a habilitation fellowship by the German Research Foundation). After being an Associate Professor in the Institute for Biology II in Freiburg from 1997-1999, he obtained a Junior Research Group by the Volkswagen Foundation, and a Full Professor of Molecular Cell Biology "at the Botanical Institute, University of Karlsruhe in 2003. After declining a call to Salzburg University, he became Head of the department in 2005 and the Botanical Garden of the KIT. He has been the Editor-in-chief of PROTOPLASMA (Springer) since 2003, since 2011 also editor of Plant Cell Monographs (Springer).

**Uli Lemmer** received the diploma degree from the RWTH Aachen University, Germany in 1990 and the Ph.D. degree from the University of Marburg, Germany, in 1995. From 1995 to 1996, he held a postdoctoral position with the University of California at Santa Barbara. He was with the University of Munich, Germany, from 1996 to 2002. In 2002, he was appointed a full Professor and director of the Light Technology Institute, Karlsruhe Institute of Technology (KIT), Germany. Uli Lemmer is currently the head of the Optoelectronics Department Director Smart Nanosystems and Printed Electronics.



Graphical Abstract