# $HgNO_3$ sensitivity of AlGaN/GaN field effect transistors functionalized with phytochelating peptides

Cite as: AIP Advances **6**, 065105 (2016); https://doi.org/10.1063/1.4953806
Submitted: 01 April 2016 . Accepted: 28 May 2016 . Published Online: 07 June 2016

Nathaniel Rohrbaugh, Luis Hernandez-Balderrama, Felix Kaess, Ronny Kirste, Ramon Collazo, and Albena Ivanisevic

# $HgNO_3$ sensitivity of AlGaN/GaN field effect transistors functionalized with phytochelating peptides

Nathaniel Rohrbaugh,[1] Luis Hernandez-Balderrama,[1] Felix Kaess,[1] Ronny Kirste,[1,2] Ramon Collazo,[1] and Albena Ivanisevic[1,a]
[1]*Department of Materials Science and Engineering, North Carolina State University, 1001 Capability Drive Raleigh, North Carolina 27606, USA*
[2]*Adroit Materials, 2054 Kildaire Farm Rd., Suite 205, Cary, North Carolina 27518, USA*

(Received 1 April 2016; accepted 28 May 2016; published online 7 June 2016)

This study examined the conductance sensitivity of AlGaN/GaN field effect transistors in response to varying $Hg/HNO_3$ solutions. FET surfaces were covalently functionalized with phytochelatin-5 peptides in order to detect Hg in solution. Results showed a resilience of peptide-AlGaN/GaN bonds in the presence of strong $HNO_3$ aliquots, with significant degradation in FET $I_D$ signal. However, devices showed strong and varied response to Hg concentrations of 1, 10, 100, and 1000 ppm. The gathered statistically significant results indicate that peptide terminated AlGaN/GaN devices are capable of differentiating between Hg solutions and demonstrate device sensitivity.  [http://dx.doi.org/10.1063/1.4953806]

There is a growing need for a broader and easily accessible set of sensing modalities that can adjust to the wide variety of environmental factors present in our modern society.[1,2] Semiconductor devices have shown great promise for these types of applications; in particular III-nitrides are becoming increasingly attractive.[3] AlGaN/GaN field effect transistors (FETs) have channel conductance that can be controlled by the presence of charged surface groups, which opens new possibilities for next generation sensing devices.[4] This behavior arises from a 2-dimensional electron gas (2DEG) present at the interface between the AlGaN and GaN and close to the device surface. Details on the working principle of AlGaN/GaN FETs have been discussed at length in previous works.[5–7] Successful efforts have been done to capture this behavior using methods such as silanization and functional group termination.[8–10] New surface treatments and functionalization techniques are being studied to further increase the utility of AlGaN/GaN and elevate them to adaptable sensing platforms.

Peptides provide a versatile choice as a surface modification due to the large variety and abundance of sensing strategies they present. One type in particular is recognition peptides, which are peptides that are highly selective to a specific compound or stimulus.[11] These are promising for creating detection profiles coupled with FETs for response within complex environmental or biological systems.[12] There will always be a need for active monitoring of pollutants such as Hg in environmental systems, and *in-situ* sensing techniques would be very advantageous.[13] The catalog of chelating and recognition peptides has expanded greatly over the last decade of research such that these sensing systems are well understood.[14,15]

In this study we utilize AlGaN/GaN FETs for Hg detection using a phytochelating peptide, Phytochelatin-5 (H-γ-EC-γ-EC-γ-EC-γ-EC-γ-ECG-OH), covalently bound to the AlGaN surface.[16] Phytochelatins were chosen due to their ability to isolate heavy metal ions, and most commonly seen in various plant species. This behavior comes from the thiol group present on each constituent glutathione group in the peptide chain, and allows for a chelate effect as the phytochelatin changes conformation around the metal ion. Prior publications have covered in depth phytochelatin

[a]Author to whom correspondence should be addressed. Electronic mail: ivanisevic@ncsu.edu

 

and associated chelate effect behavior.[17,18] Devices were tested with varying concentrations of Hg, with results monitoring changes in the device sensitivity and performance. Effects from nitric acid ($HNO_3$) concentration in solution to AlGaN/GaN FETs are reported in this work. $HNO_3$ is commonly present in Hg standard solutions to better facilitate dissolution of Hg. Functionalization of the sensing devices was done using a phosphoric acid and ethephon:phosphonic acid etching procedure which covalently binds peptide chains to the FET surfaces.[19,20]

AlGaN/GaN FETs were fabricated using methods previously described.[21] Finished wafers were diced into 3 mm x 3 mm chips each containing up to 13 FETs giving a total of 40 individual chips used in each group of conditions described in this study. Approximately 500 FETs were tested in total. All FETs were tested for current-voltage (IV) characteristics and response after each step in the functionalization and Hg exposure process. The phosphoric acid ($H_3PO_4$) and ethephon ($C_2H_6ClO_3P$) functionalization was done following previously discussed procedures.[20] Peptides used in this study were purchased from Anaspec, EGT; 1000 ppm Hg in 9% $HgNO_3$ standard purchased from LabChem. Functionalized chips were then dried over 48 hours with 10 μL solutions of Hg. The following concentrations were tested: 1000 ppm, 100 ppm, 10 ppm, 1 ppm, and 0 ppm of Hg. Confirmation of surface modification and Hg presence was done using x-ray photoelectron spectroscopy (XPS). IV results were monitored by a percent change in drain current ($I_D$) at a constant drain voltage ($V_D$) and were taken using a Keithley 4200-SCS using KITE v8.2 attached to a probe station. XPS measurements were done using a Kratos Analytical Axis Ultra XPS and CasaXPS v2.3. Data analysis, plotting, and statistical calculations were performed using Origin. Figure 1 shows a schematic of the acid surface functionalization (a), the binding between peptide chains and the FET surface (b), and the exposure of the device to Hg solution (c).

Throughout the testing process, samples were removed for XPS analysis in order to confirm the presence of modifications generated during the functionalization process.[22] Peptide functionalized surfaces showed evidence of strong amide peaks as seen in Figure 2 at 399.8 eV.[23,17] These peaks persisted throughout testing with the addition of the various Hg solutions. This can also be seen in Figure 2 within the 10 and 1000 ppm line scans. This is in agreement with previous work in testing the covalent bonding of peptides to the surface and shows surface peptides remaining intact after exposure to varying solutions of $HNO_3$.[11] Diminishing Hg 4f peaks at 104.7 and 101.3 eV were also observed with subsequent steps in the Hg solution used for the various FET test groups, where the peak locations were cross referenced with NIST XPS database.[18] $HNO_3$ within the Hg standard solution can also be observed as a nitrate peak (N 1s at 407.5 eV) present in the 1000 ppm and 10 ppm line scans.[24] With regards to the samples used in this study these XPS scans provided qualitative confirmation of the presence of both TAT-C and Phytocelatin-5 peptides before moving into IV characterization of the devices.

Initial results from the IV testing revealed solution effects that could not be attributed to Hg-peptide interactions on the AlGaN/GaN surfaces. Cleaned control samples tested in water showed little change throughout the course of this study and especially when compared to the etch treatments used. Etching of the surface done in the HCl and following phosphonic acid:ethephon etch resulted in a loss of signal or $I_D$ of an average 10-30%, as seen in Figure 3(a).[25] Comparing this to the untreated control in water there is a statistically significant effect of this functionalization effect upon the devices. This is consistent with work previously done by this group which showed

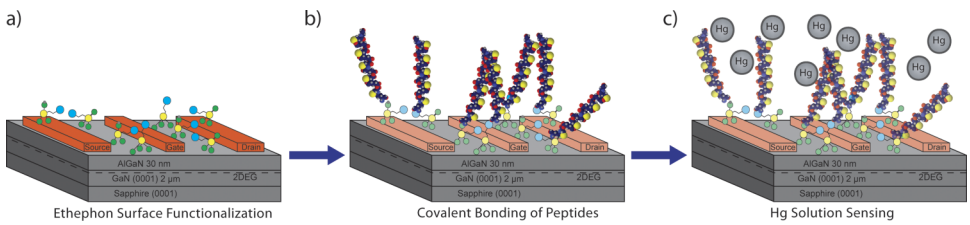


FIG. 1. a) Ethephon and phosphoric acid surface functionalization, b) Peptide incubation resulting in covalent binding between peptide chains and AlGaN/GaN FET surfaces, c) Hg standard solution exposure to Hg-sensitive peptide functionalized AlGaN/GaN FETs.

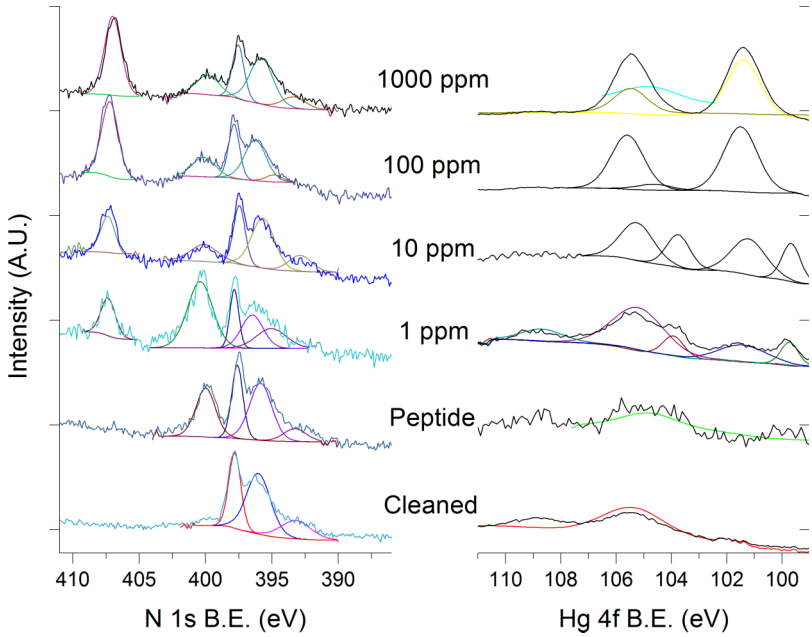


FIG. 2. XPS spectra of cleaned surfaces, peptide functionalized, 1, 10,100 and 1000 ppm Hg confirming lack of amide and Hg peaks prior to the functionalization steps and affirming the diminishing Hg peaks as concentration was stepped down.

a similar effect using only TAT-C functionalization to demonstrate covalent bonding.[19] A similar effect appears to be caused by the Hg solution itself. Testing done with the undiluted Hg standard solution at 1000 ppm resulted in a significant drop in FET activity of an average 60-80% which is shown in Figure 3(a). This is attributed to $HNO_3$ in solution degrading the Au contacts and metal stack that makes up the gate and source/drain features along the FET surfaces. Sensing using phytochelating peptides occurs due to the conformation and charge changes present along the AlGaN/GaN surface and not the Au contacts, thus this behavior is unaffected.[15] However, any surface or FET contact damage from unwanted etching disrupts the surface charge sensitivity that is created at the AlGaN/GaN interface and hinders the efficacy of the sensor. By testing for these effects, we isolated and then validated the biomolecular effects observed between the Hg within solution and the Phytochelatin-5 peptide. However, it is believed that these degradation effects can be minimized by directly protecting the contacts during the soaking step or using an optimized FET design with remote contacts. The effect of the Hg standard degrading FET signal can be seen in the ethephon functionalized surfaces in both Figure 3(a) and Figure 3(b). Further examination of the controls group showed this effect to be unrelated to the addition of the Phytochelatin-5 peptide chain.

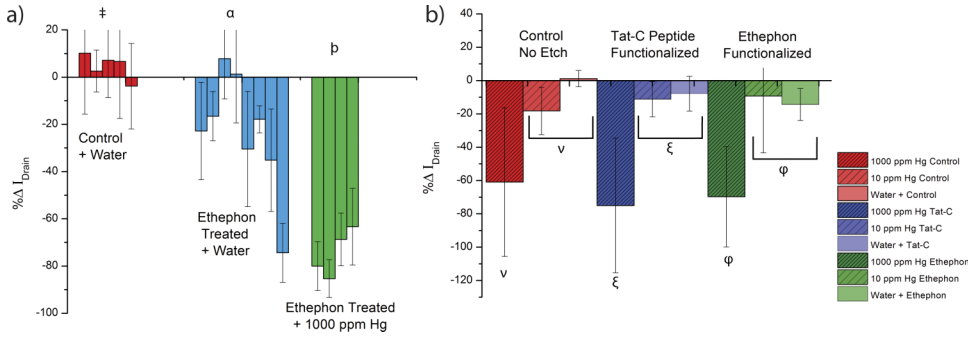


FIG. 3. a) Three control groups testing the effects of the different solution treatments performed in this experiment including a water control, full ethephon functionalization, and then full functionalization plus the undiluted Hg stock solution. b) Comparison of water, 1000 ppm, and 10 ppm effects on functionalized samples and the negative peptide control using TAT-C.

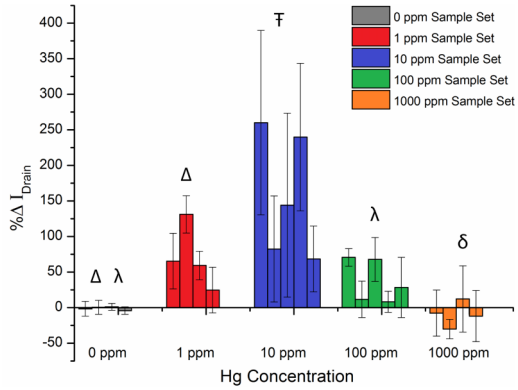


FIG. 4. $I_D$ response of phytochelatin modified AlGaN/GaN FETs exposed to varying concentrations of Hg in nitric acid solution. Each bar represents a single chip containing a maximum of 13 individual FETs. Significant differences exist between these groups indicating a sensitivity to varying Hg concentration within the solution.

FETs measuring 10 ppm were also examined in these earlier IV scans in testing for degradation or interaction effects. This data, visible in Figure 3(b), did not show any clear effect or statistically significant effect on control or peptide samples. In this figure, 10 ppm controls are compared alongside 1000 ppm and show a lack of the 60-80% degradation seen in the 1000 ppm samples. Thus IV measurements concluded that the ethephon functionalization and Hg standard solution itself negatively impact FET conductance properties to varying degrees. More so the TAT-C peptide control did not yield any recognition effects as seen later by Phytochelatin-5 treated samples.

When testing for the Hg in solution, the Phytochelatin-5 was chosen as a known peptide that is sensitive to Hg.[26,27] This effect was indeed observed upon our FETs in this experiment as well, and with varying sensitivities. As can be seen in Figure 4 there is a statistically significant difference when moving along the concentration gradient of samples tested from 0 ppm up to 1000 ppm. Immediately visible is that the 1000 ppm coated Phytochelatin-5 sample set does not show the same 60-80% drop off in $I_D$ noted in prior control samples. In fact, as concentration decreases there is a significant increase in the $I_D$ measurements up to a maximum of 10 ppm before dropping once again when approaching 1 ppm. In referencing previous scans shown in Figure 3(b) the addition of Hg solutions only reduced FET signal strength and can be seen in the majority of the control samples. However, a large and significant positive percent difference can be observed between those control tests and samples that were incubated in the Phytochelatin-5 peptide. The interaction between Hg in solution and Phytochelatin-5 appears to be occurring and is quantifiable by these FET devices. Varying sensitivities noted in these Phytochelatin-5 samples suggests that this incubation method of peptide based sensing molecules covalently bound to a surface is capable of detecting varying sensitivities of a specific compound or element in solution. One possible explanation for the lack of increasing signal in the 100 ppm and 1000 ppm is the same degradation effect brought on by the $HNO_3$ as observed in control samples in Figure 3(a) and 3(b).[28] The resulting data then suggests either a passivation or compensation by the peptide adlayer itself. Aside from these two sample sets, which already behaved significantly differently than controls, the recognition events are clearly visible in the remaining two Hg sample sets of 10 ppm and 1 ppm.

AlGaN/GaN FETs were found to provide concentration sensitive behavior using the ethephon-peptide binding scheme presented here. Hg was selected as a known neurotoxic polluting compound that would be of possible interest to future environmental sensors. Other heavy metal systems, such as Cr, and Pb, with issues of bioaccumulation exist and provide opportunities for testing in the future.[29] The stability of the sensing layer in the presence of aqueous solutions makes this technique viable for future exploration under more representative in-field testing. Data shown here has demonstrated that the covalently bound peptides to AlGaN/GaN surfaces were still capable of producing a recognition effect in the presence of a 9% solution of $HNO_3$. In addition to this stability, surfaces demonstrated sensitivity to varying Hg concentrations. This will eventually allow for highly sensitive heavy metal sensors for future devices that can take advantage of the AlGaN/GaN stability

and chemical inertness. Future work in this field would suggest investigation into selectivity of a functionalized AlGaN/GaN FET when exposed to a more complex solution system.

## ACKNOWLEDGEMENTS

We thank Army Research Office under W911NF-15-1-0375 for support of this work.

[1] M. Stutzmann, G. Steinhoff, M. Eickhoff, O. Ambacher, C. E. Nebel, J. Schalwig, R. Neuberger, and G. Müller, "GaN-based heterostructures for sensor applications," Diamond and Related Materials **11**(3–6), 886-891 (2002).
[2] R. Kirste, N. Rohrbaugh, I. Bryan, Z. Bryan, R. Collazo, and A. Ivanisevic, "Electronic Biosensors Based on III-Nitride Semiconductors," Annual Review of Analytical Chemistry **8**, 149-169 (2014).
[3] E. Estephan, C. Larroque, F. d. r. J. G. Cuisinier, Z. n. Bálint, and C. Gergely, "Tailoring GaN Semiconductor Surfaces with Biomolecules," The Journal of Physical Chemistry B **112**(29), 8799-8805 (2008).
[4] B. Baur, J. Howgate, H. G. von Ribbeck, Y. Gawlina, V. Bandalo, G. Steinhoff, M. Stutzmann, and M. Eickhoff, "Catalytic activity of enzymes immobilized on AlGaN/GaN solution gate field-effect transistors," Applied Physics Letters **89**(18), 3901 (2006).
[5] M. S. Makowski, S. Kim, M. Gaillard, D. Janes, M. J. Manfra, I. Bryan, Z. Sitar, C. Arellano, J. Xie, R. Collazo, and A. Ivanisevic, "Physisorption of functionalized gold nanoparticles on AlGaN/GaN high electron mobility transistors for sensing applications," Applied Physics Letters **102**(7), 074102 (2013).
[6] T. R. Lenka and A. K. Panda, "Characteristics study of 2DEG transport properties of AlGaN/GaN and AlGaAs/GaAs-based HEMT," Semiconductors **45**(5), 650-656 (2011).
[7] O. Ambacher, B. Foutz, J. Smart, J. R. Shealy, N. G. Weimann, K. Chu, M. Murphy, A. J. Sierakowski, W. J. Schaff, L. F. Eastman, R. Dimitrov, A. Mitchell, and M. Stutzmann, "Two dimensional electron gases induced by spontaneous and piezoelectric polarization in undoped and doped AlGaN/GaN heterostructures," Journal of Applied Physics **87**(1), 334-344 (2000).
[8] B. H. Chu, C. Y. Chang, K. Kroll, N. Denslow, Y.-L. Wang, S. J. Pearton, A. M. Dabiran, A. M. Wowchak, B. Cui, P. P. Chow, and F. Ren, "Detection of an endocrine disrupter biomarker, vitellogenin, in largemouth bass serum using AlGaN/GaN high electron mobility transistors," Applied Physics Letters **96**(1), 013701 (2010).
[9] A. Podolska, M. Kocan, A. M. G. Cabezas, T. D. Wilson, G. A. Umana-Membreno, B. D. Nener, G. Parish, S. Keller, and U. K. Mishra, "Ion versus pH sensitivity of ungated AlGaN/GaN heterostructure-based devices," Applied Physics Letters **97**(1), 012108 (2010).
[10] M. Myers, F. L. M. Khir, A. Podolska, G. A. Umana-Membreno, B. Nener, M. Baker, and G. Parish, "Nitrate ion detection using AlGaN/GaN heterostructure-based devices without a reference electrode," Sensors and Actuators B: Chemical **181**, 301-305 (2013).
[11] N. Rohrbaugh, I. Bryan, Z. Bryan, C. Arellano, R. Collazo, and A. Ivanisevic, "AlGaN/GaN field effect transistors functionalized with recognition peptides," Applied Physics Letters **105**(13), (2014).
[12] P. Casal, X. Wen, S. Gupta, T. Nicholson, Y. Wang, A. Theiss, B. Bhushan, L. Brillson, W. Lu, and S. C. Lee, "ImmunoFET feasibility in physiological salt environments," Philosophical Transactions of the Royal Society of London A: Mathematical, Physical and Engineering Sciences **370**(1967), 2474-2488 (2012).
[13] F. He, J. Gao, E. Pierce, P. J. Strong, H. Wang, and L. Liang, "In situ remediation technologies for mercury-contaminated soil," Environmental Science and Pollution Research **22**(11), 8124-8147 (2015).
[14] V. Shankar, V. Thekkeettil, G. Sharma, and V. Agrawal, "Alleviation of heavy metal stress in Spilanthes calva L. (antimalarial herb) by exogenous application of glutathione," In Vitro Cellular & Developmental Biology - Plant **48**(1), 113-119 (2011).
[15] W. Ben Ammar, C. Mediouni, B. Tray, M. H. Ghorbel, and F. Jemal, "Glutathione and phytochelatin contents in tomato plants exposed to cadmium," Biologia Plantarum **52**(2), 314-320.
[16] V. Diopan, V. Shestivska, O. Zitka, M. Galiova, V. Adam, J. Kaiser, A. Horna, K. Novotny, M. Liska, L. Havel, J. Zehnalek, and R. Kizek, "Determination of Plant Thiols by Liquid Chromatography Coupled with Coulometric and Amperometric Detection in Lettuce Treated by Lead(II) Ions," **- 22**(- 11), - 1259(2010).
[17] L. P. Wei, J. R. Donat, G. Fones, and B. A. Ahner, "Interactions between Cd, and Cu, and Zn influence particulate phytochelatin concentrations in marine phytoplankton: Laboratory results and preliminary field data," Environ. Sci. Technol. **37**(16), 3609-3618 (2003).
[18] F. Wu and G. Zhang, "Phytochelatin and its function in heavy metal tolerance of higer plants," Yingyong Shengtai Xuebao **14**(4), 632-636 (2003).
[19] N. Rohrbaugh, I. Bryan, Z. Bryan, R. Collazo, and A. Ivanisevic, "Long-term stability assessment of AlGaN/GaN field effect transistors modified with peptides: Device characteristics vs. surface properties," AIP Advances **5**(9), 097102 (2015).
[20] N. G. Berg, M. W. Nolan, T. Paskova, and A. Ivanisevic, "Surface Characterization of Gallium Nitride Modified with Peptides before and after Exposure to Ionizing Radiation in Solution," Langmuir **30**(51), 15477-15485 (2014).
[21] S. Mita, R. Collazo, A. Rice, R. F. Dalmau, and Z. Sitar, "Influence of gallium supersaturation on the properties of GaN grown by metalorganic chemical vapor deposition," Journal of Applied Physics **104**(1), (2008).
[22] C. M. Foster, R. Collazo, Z. Sitar, and A. Ivanisevic, "Cell Behavior on Gallium Nitride Surfaces: Peptide Affinity Attachment versus Covalent Functionalization," Langmuir **29**(26), 8377-8384 (2013).
[23] M. S. Makowski, D. Y. Zemlyanov, J. A. Lindsey, J. C. Bernhard, E. M. Hagen, B. K. Chan, A. A. Petersohn, M. R. Medow, L. E. Wendel, D. Chen, J. M. Canter, and A. Ivanisevic, "Covalent attachment of a peptide to the surface of gallium nitride," Surface Science **605**(15–16), 1466-1475 (2011).
[24] B. L. Pearce, N. G. Berg, M. S. Rahn, and A. Ivanisevic, "In situ and ex situ functionalization of nanostructured gallium oxy-hydroxide with a porphyrin dye" (2016) - n/a..

[25] M. Reiner, M. Reiss, T. Brünig, L. Knuuttila, R. Pietschnig, and C. Ostermaier, "Chemical understanding and utility of H3PO4 etching of group-III- nitrides," Phys. Status Solidi B **252**(5), 1121-1126 (2015).
[26] L. Chen, L. Yang, and Q. Wang, "In vivo phytochelatins and Hg-phytochelatin complexes in Hg-stressed Brassica chinensis L," Metallomics **1**(1), 101-106 (2009).
[27] A. Dago, C. Arino, J. M. Diaz-Cruz, and M. Esteban, "Analysis of phytochelatins and Hg-phytochelatin complexes in Hordeum vulgare plants stressed with Hg and Cd: HPLC study with amperometric detection," Int. J. Environ. Anal. Chem. **94**(7), 668-678 (2014).
[28] C. Bo-Yi, H. Wei-Chou, L. Ching-Sung, L. Han-Yin, T. Chih-Ming, and H. Chiu-Sheng, "Investigations of AlGaN/GaN HFETs utilizing post-metallization etching by nitric acid treatment," Semiconductor Science and Technology **28**(7), 074003 (2013).
[29] H. P. Jambhulkar and A. A. Juwarkar, "Assessment of bioaccumulation of heavy metals by different plant species grown on fly ash dump," Ecotox. Environ. Safe. **72**(4), 1122-1128 (2009).