

Research Article

Open Access

High Spatial Resolution Chemical Imaging of Inorganic and Organometallic Pesticides

Richard Ortega^{1,2*}, and Asuncion Carmona^{1,2}

¹Univ. Bordeaux, CENBG, UMR 5797, F-33170 Gradignan, France

²CNRS, IN2P3, CENBG, UMR 5797, F-33170 Gradignan, France

Abstract

The recent development of high spatial resolution analytical methods for inorganic element imaging enables the sensitive determination of inorganic and organometallic-based pesticides, at the cellular and subcellular levels. Some organic agrochemicals can also be investigated providing they contain one or more hetero-element, such as the halogens fluorine or bromine. Several micro-chemical imaging methods are currently available and will be reviewed in this article: Electron Microscopy combined to Energy Dispersive X-ray Spectrometry (EM-EDS), PIXE (Particle Induced X-ray Emission), SXRF (Synchrotron Radiation X-ray Fluorescence) combined to micro-XAS (X-ray Absorption Spectroscopy), and Secondary Ion Mass Spectrometry (SIMS). Although these methods offer a high potential in the understanding of the cellular mechanisms of pesticides toxicity, they have not been much used yet in this domain of investigation. The aim of this article is to make known these imaging methods to the scientific community interested in the toxicology of pesticides. Some examples of applications taken from our own investigations, and from other researchers, will be presented to illustrate the kind of information can be gathered from these methods. In particular, the subcellular distribution of Maneb in neuronal cells will be discussed, as well as the subcellular chemical speciation of arsenic in human ovarian cells.

Introduction

Some pesticide chemicals are based on inorganic elements such as sulfur, copper (i.e. copper sulfate '*Bordeaux mixture*', copper hydroxide, and copper oxychloride), and arsenic salts (i.e. lead arsenate, calcium arsenate, sodium arsenite), or they contain an inorganic element in their chemical structure such as the organometallic pesticides based on manganese dithiocarbamate (maneb), manganese and zinc dithiocarbamate (mancozeb), organotin compounds (triphenyltin, tributyltin, and tricyclohexyltin), herbicide MSMA (monosodium methyl arsenate), or the brominated compounds deltamethrin and bromoxynil. Using state-of-the art micro-analytical methods, inorganic elements can be probed with high spatial resolution, and high sensitivity, to reveal their tissue and cell distribution to understand their mechanisms of toxicity at their site of action.

A variety of techniques exist that provide chemical information in the form of a spatially resolved image [1,2]. These methods provide information on the spatial distribution of trace elements within solid samples with at least a micrometer resolution. The following article gives an overview of the principle and the performance of the main micro-analytical techniques used in inorganic element determination. Their analytical characteristics are summarized and compared in Table 1. The article will also review their application in the field of pesticide toxicology. These micro analytical techniques provide detailed information concerning the distribution, at the tissue and cell levels of inorganic elements and are very valuable as a complement to the more conventional bulk analysis. They have been used in plant studies leading to significant breakthroughs in understanding the role of physiological metals, such as for example in the case of Fe metabolism by revealing sometimes unexpected sites of sub-cellular location [3,4]. Up to now these methods have not been frequently applied in the field of pesticide toxicology. For each of these micro-analytical methods their principle will be briefly presented and their applications in the field of pesticide toxicology will be reviewed.

Electron Microscopy with Energy Dispersive X-ray Spectroscopy (EM-EDS)

Principle

EM-EDS is a well-established and affordable technique with which it is possible to determine the elemental composition of a specimen in the electron microscope. When an electron beam irradiates a specimen, a number of different interactions occur, and particularly, X-rays are generated as a consequence of the rearrangement of outer shell electrons after an inner shell electron has been ejected from the atom. When the energy of the incident electrons is higher than the electron binding energy from the inner shell, ejection of these inner shell electrons occurs and results in outer-shell electrons filling the vacancies, and hence emitting X-rays. The energy of the emitted X-rays is characteristic of the excited element. It corresponds to the difference between the two electronic levels binding energies. The emission energies of all chemical elements are tabulated thus enabling the identification of the composition of the atoms constitutive of the sample. Emitted X-rays carry information about the elemental composition of the specimen in the region that is being irradiated. An excellent and comprehensive description of scanning electron microscopy including EM-EDS is given in the textbook of Goldstein *et al.* [5]. X-ray emission spectroscopy enables multi-element determination with simultaneous detection of all elements of $Z > 5$ using low energy-dispersive X-ray detectors. Microscopic examination using EM and energy dispersive

***Corresponding author:** Richard Ortega, University of Bordeaux, CENBG, UMR 5797, F-33170 Gradignan, France, E-mail: ortega@cenbg.in2p3.fr

Received April 02, 2012; Accepted April 21, 2012; Published April 23, 2012

Citation: Ortega R, Carmona A (2012) High Spatial Resolution Chemical Imaging of Inorganic and Organometallic Pesticides. J Environ Anal Toxicol S4:005. doi:10.4172/2161-0525.S4-005

Copyright: © 2012 Ortega R, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Spatially resolved analytical techniques	Detection limit	Spatial resolution	Selectivity	Quantification	Analytical depth
EDS (X-ray Energy Dispersive Spectrometry)	100 to 1000 µg/g	0.5 µm	Multielemental (Z≥6)	Quantitative	0.1 – 1 µm
PIXE (Particle Induced X-ray emission)	1 to 10 µg/g	0.2 – 2 µm	Multielemental (all Z)	Quantitative	10 - 100 µm
SXRF (Synchrotron radiation X-ray fluorescence)	< 0.1 µg/g	0.1 – 2 µm	Multielemental (Z≥6)	Quantitative	>100 µm
XAS (X-ray Absorption Spectroscopy)	100 µg/g	1 µm	Chemical species	Quantitative	>100 µm
Secondary Ion Mass Spectrometry (SIMS)	0.1 µg/g	0.05 µm	Multielemental and Isotopic	Semi-quantitative	0.1 µm

Table 1: Main spatially resolved analytical techniques for chemical element imaging, quantification, and speciation in biological samples (adapted from [1]).

X-ray analysis of chemical elements can be combined allowing the high spatial resolution morphological and chemical characterization of samples. Compared to other micro-analytical methods the main drawback of EM-EDS is probably its limited sensitivity (mg/g), however, it is a widely available and affordable method that should be considered first at the time to carry out micro-analytical investigations.

EM-EDS applications in pesticide toxicology

Early work on fungicide penetration within leaves showed that the chemical elements constitutive of some specific pesticides could be probed to investigate their presence and penetration [6]. In a more recent study, EM-EDS proved to be a simple and useful method for the quantitative determination of agrochemicals at the plant surface [7]. The conclusions of this study highlighted that a reliable quantification could be obtained for the inorganic copper compounds (copper hydroxide and copper oxychloride), the sulfur agrochemicals, the organometallic compound mancozeb, and also for organic pesticides such as glyphosate, glufosinate, and bromoxynil. These late were detected and quantified using the halogen element Cl or Br as markers. However, to ensure reliable quantitative results, the use of an internal standard such as calcium nitrate was advocated by the authors and caution must be taken about the possible interference of this internal standard with the penetration of the pesticide. When compared to more conventional bulk analysis of pesticides in plants, EM-EDS offers a spatially resolved determination. However the analysis is limited to the surface of the leaves rendering difficult the application of EM-EDS to intracellular determination. This is probably why there are only very few publications of EM-EDS applications in this field of investigation.

PIXE (Particle Induced X-ray Emission)

Principle

The focusing of MeV ion beams (H^+ , He^+) down to small areal dimensions, typically a few μm^2 , enables the application of PIXE analysis to microscopic regions of a sample. PIXE is based on the detection of characteristic X-rays emitted by the elements of a sample when excited with a MeV ion beam produced by a particle accelerator. It is generally performed with protons, better than with heavier particles, because the ionization cross sections follow an A^{-4} law, where A is the atomic weight of the incoming particle. PIXE is a multielemental technique which offers a good sensitivity for elements with atomic numbers of $Z > 11$. The physical processes involved in the interaction between the particle beam and the electronic shells of the atoms have been extensively described making PIXE a standardless quantification technique. The detection limits depend on the considered element, the nature of the sample, the experimental set-up and the analytical conditions. Detection limits are usually in the $\mu g/g$ range. The main advantages of the PIXE method, compared to EM-EDS, are its higher sensitivity and higher penetration depth. PIXE technique presents greater ionization cross sections and lower background signal than electron x-ray microanalysis. By using a proton beam instead of an electron beam, micro-analytical determination can be performed with a sensitivity which is 100 times greater than of EM-EDS. Another important advantage over the other

X-ray microanalytical techniques is that fully quantitative results are obtained thanks to the simultaneous analysis of trace elements using PIXE, and sample mass using proton BS and STIM [8]. The ion microbeam can be scanned over the surface of the specimen and thus provide information on the lateral distribution of elemental species. Ion microprobes are often called nuclear microprobes which involve the distinct micro-spectrometries that can be performed depending on the ion beam effect of interest, such as PIXE, but also elastic recoil detection analysis (ERDA), Rutherford backscattering spectrometry (RBS), ionoluminescence, or particle induced gamma emission (PIGE) nuclear reaction spectrometry. These techniques are complementary to micro-PIXE as they can be applied to light elements determination. Therefore all Z elements can be determined using ion beam analysis methods. For more detailed description of PIXE micro-analysis and applications to biological samples investigation the text book from Llabador and Moretto [9], can be consulted.

PIXE applications

Micro-PIXE has been successfully applied to study metallic stress in plants: cesium in *Arabidopsis thaliana* [10], arsenic in lichen *Hypogymnia physodes* [11], or uranium in plants [12]. There are not yet any reports using micro-PIXE to probe directly the effects of inorganic or organometallic based pesticides on biological samples. However the studies on metal stress can illustrate the potential of this technique. For example, in the study of Cs distribution in *Arabidopsis thaliana*, the multielemental capabilities of micro-PIXE enabled to confirm competition mechanisms between Cs and K, and to reveal tissue specific alteration of Mn, Fe and Zn homeostasis which could explain in part Cs phytotoxicity [10]. In the case of toxic effects of arsenic on lichens [11], micro-PIXE revealed the redistribution of most physiological trace elements (Figure 1). K and Ca were depleted from all lichen layers; Mn was depleted from the algal layer and lower cortex, Fe was decreased in the algal layer and increased in the lower cortex, Zn was decreased both in the algal layer and the medulla, but increased in the lower cortex, P and S were decreased in the medulla but increased in the algal layer and the lower cortex Mn and As in the medulla in As exposed lichens. This example illustrates how the multielemental capabilities of micro-PIXE can reveal inter-metallic effects and unexpected element redistributions. In a more recent study, the impact of uranium speciation on uranium distribution in oilseed rape, sunflower and wheat was evaluated by a combination of analytical methods including micro-PIXE and EM-EDS [12]. Micro-PIXE enabled to determine U distribution in leaves, where U concentration was too low to be detected by EM-EDS. PIXE results showed that U content was more important in sunflower than in wheat and oilseed rape, and was mainly concentrated on the epidermis region. Another related example of application is the study of insects impacted by plant pollution such in the case of zinc toxicity towards grasshoppers [13]. Zinc distribution was determined in brains from grasshoppers, *Chorthippus brunneus*, living in zinc polluted vs. control areas. Before the results of PIXE mapping were obtained, the authors expected that zinc would increase in the brain of zinc exposed animals but it was found that no simple relations between Zn contents in the brain and its concentration in the sand existed. The concentration

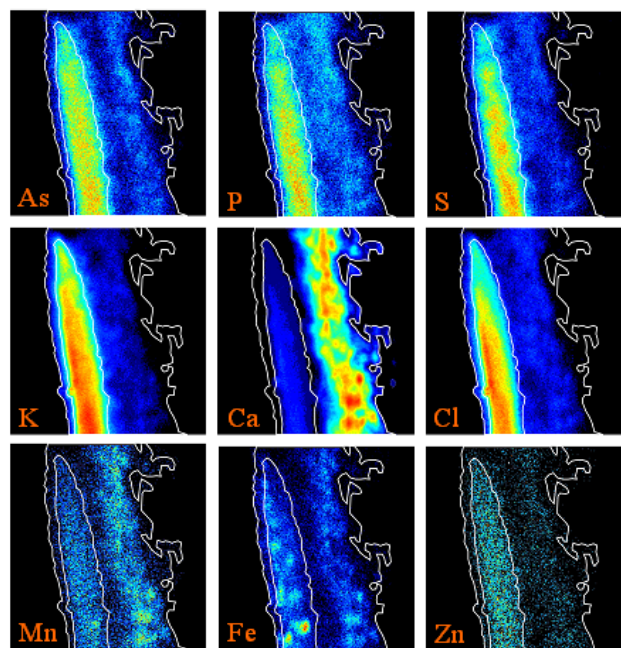


Figure 1: Yield element maps ($204 \times 204 \mu\text{m}^2$) obtained from micro-PIXE analyses of arsenic-exposed lichen thallus of *Hypogymnia physodes*. The upper portion of the lichen thallus (upper cortex, algal layer and medulla, from left to right) is shown in detail. Maps are not corrected for sample thickness. The majority of elements are concentrated in the algal layer, except for calcium and manganese that are concentrated in the medulla. Peak manganese concentration in the medulla occurred due to arsenic-exposure. Reprinted with permission from [11].

of zinc in the brain of grasshoppers representing highly Zn polluted group was lower than in the control group suggesting the involvement of specific metal rejection mechanisms. This result may be the basis for further searching for molecular mechanisms of defense against heavy metals in insects living in polluted habitats.

SXRF (Synchrotron radiation X-ray Fluorescence) and XAS (X-ray Absorption Spectroscopy)

Principle of SXRF

Another excellent excitation source to perform X-ray analysis is the synchrotron radiation. Similarly to that of PIXE, the principle of XRF is based upon the detection of X-rays emitted from sample atoms irradiated with X-rays of higher energy instead of ions. XRF analysis is multielemental and quantitative; the surface of the fluorescence peaks is directly proportional to the concentration of the elements within the sample. Third generation synchrotron sources are bright enough to generate high flux micro-beams which can be applied to map trace element distributions in biological samples at the cellular scale. However, the spatial resolution offered by SR-XRF technique was limited until recently to approximately $1 \mu\text{m}$. Improvements in hard x-rays ($> 1 \text{ keV}$) optics have been made over the last few years. Sub-micron spatial resolution X-ray fluorescence beamlines have been recently developed at the APS (Advanced Photon Source) and ESRF (European Synchrotron Radiation Facility) enabling to focus keV photons down to 100 nm beam size. The large penetration depth of hard X-rays do not require specimens to be sectioned, cells can be investigated close to their natural, hydrated state with dedicated cryogenic approaches, and without introduction of artificial dyes. Compared to ion and EM-EDS,

synchrotron radiation X-ray microbeams possess a higher penetration depth, up to $1000 \mu\text{m}$ vs. $100 \mu\text{m}$ with micro-PIXE, and $5 \mu\text{m}$ with EM-EDS (Table 1). SXRF is more sensitive than PIXE or EM-EDS, below the $\mu\text{g/g}$, which is a major advantage at to detect trace amounts of pesticides.

Principle of XAS

Another important feature of synchrotron-based methods is their ability to operate spatially resolved chemical speciation analysis, thanks to XAS. X-Ray absorption spectra are obtained by tuning the photon energy around the absorption edge of a specific element and by measuring the photons transmitted through the sample. Each core electron has a well-defined binding energy, and when the energy of the incident x-ray is scanned across one of these energies, there is an abrupt increase in the absorption coefficient. Local structural information on the absorber element can be deduced by analyzing oscillations in X-ray absorption versus photon energy that are caused by the scattering of the X-ray excited photoelectron. XAS can be divided into X-ray absorption near edge structure (XANES), which provides information primarily about geometry and oxidation state, and extended X-ray absorption fine structure (EXAFS), which provides information about metal site ligation.

SXRF applications: Maneb neurotoxicology

We have recently investigated in our group the neurotoxicology of the fungicide Maneb using SXRF at the European Synchrotron Radiation Facility (ESRF). The aim of this experiment was to determine metal distribution in rat dopaminergic PC12 cells when exposed to Maneb, a manganese based organometallic pesticide (*1,2-ethanediyl bis(carbamodithioato)(2-))manganese*). We were especially interested in determining the cellular distribution of manganese in dopamine producing cells because Maneb could be involved in the aetiology of Parkinson's disease [14]. In this experiment, a setup for high spatial resolution X-ray fluorescence microanalysis based on a multilayer lens and a piezoelectric sample stage was used on ID21 beamline at ESRF, thus leading to the obtention of a photon beam with $0.27 \mu\text{m} \times 1.3 \mu\text{m}$ ($v \times h$) spatial resolution and with 10^9 ph/s flux of photons. SXRF of rat dopaminergic PC12 cells enabled to define the subcellular distribution of Mn and essential trace elements such as P, S, Cl and K (Figure 2). In PC12 cells exposed to MnCl_2 , Mn accumulates in a specific region of cell cytosol, near the nucleus, identified as the Golgi apparatus in a previous study [15]. In figure 2, a similar distribution was observed showing the same peri-nuclear accumulation of manganese of cells are exposed to Maneb instead of MnCl_2 . This unexpected cellular localization of Mn suggests that high concentrations of Mn could alter Golgi apparatus functions resulting in a defective trafficking of neurosecretory vesicles (synthesized by Golgi apparatus) and an altered storage of dopamine in

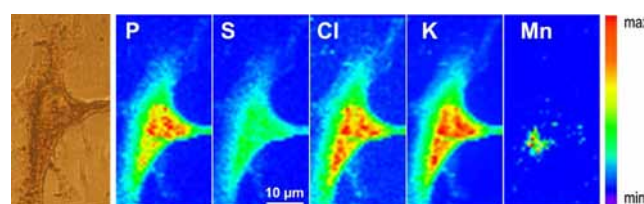


Figure 2: Elemental distributions in a PC12 cell exposed to $50 \mu\text{M}$ of Maneb during 24 hours. Scan size is $32 \times 64 \mu\text{m}$. At left: image of the cell before irradiation and at right: x-ray fluorescence images. Color bar ranges from blue to red (min to max) is proportional to the number of X-rays detected.

cells explaining the specificity of dopaminergic cells neurodegeneration in Parkinson's disease.

XAS applications. Arsenic speciation

Although the use of arsenic compounds as pesticides is declining, arsenic inorganic pesticides are persistent pollutants and well-documented carcinogens, even at low concentration. The precise mechanisms of arsenic cancer causing effects are unknown. The identity of arsenic species involved in carcinogenesis is ambiguous and metabolites of inorganic species seem to create more toxic arsenic compounds by methylation. In an attempt to understand the cellular mechanisms of As carcinogenesis we performed micro-XANES on human ovarian cells (IGROV1) exposed to inorganic arsenic [16]. Mitochondria have been suggested to be a target organelle for As toxicity. Mitochondria were marked with a fluorescent dye (Rhodamine123) and their intracellular location was determined by confocal microscopy. XANES experiments were performed with a $1.5 \times 4.0 \mu\text{m}^2$ spatial resolution using ID-22 hard X-ray microprobe with a Kirkpatrick-Baez focusing mirror at ESRF. This experimental setup provided a photon flux of typically 1.5×10^{11} ph/s allowing X-ray absorption spectroscopy to be performed with micrometric resolution. The use of a liquid nitrogen cryo-jet enabled to analyze the samples in their frozen hydrated state at 150 K. Arsenic chemical speciation was determined locally, by point analysis, into nucleus, mitochondria and cytoplasm by scanning around As absorption K-edge from 11860 to 11910 eV with 0.5 eV energy resolution. An example of μ -XANES spectra within cell compartments (mitochondria, nucleus, and cytosol) is presented in figure 3. Arsenic oxidation state was found similar in each cells compartment. μ -XANES analyses of As chemical standards shown that As absorption edge in cell compartments was between those of As_2O_3 standard and dimethyl-As (V) standard (DMAV), which were separated by less than 1 eV. However μ -XANES did not allow the formal identification of As chemical forms (mixture of both compounds?). This experiment has opened a new field of investigation, the in-situ determination of chemical element oxidation states within cellular organelles.

Secondary Ion Mass Spectrometry (SIMS)

The SIMS technique is based on the interaction of primary ions (Ar^+ , Xe^+ , O_2^+ , Cs^+ ,...) in the keV range with the surface components of solids. The ion beam is scanned over the sample surface in order to sputter the first external atomic layers. Atoms, or clusters of atoms, can be emitted either in a neutral or charged state. The secondary ions are then directed towards a mass spectrometer and analyzed according to their m/z ratio. SIMS provides imaging capabilities of element distributions and has been used with different types of mass analyzers, such as quadrupoles or time-of-flight analyzers. SIMS is multielemental with isotopic capability, characterized by high detection limits of 0.1 to $1 \mu\text{g/g}$ in element imaging mode, and high spatial resolution of typically 50 nm generally employed for cellular imaging [17]. SIMS analyses are divided into two broad categories known as dynamic and static. In the dynamic type, the most common SIMS method, a relatively intense primary ion beam sputters the sample surface at high sputter rates, providing a very useful way to determine the in-depth concentration of different elements in a solid. The most common secondary ions detected in a dynamic secondary ion mass spectrometry analysis are elemental ions or clusters of elemental ions. When used in dynamic mode, SIMS is quantitative; the local organic mass content can be determined. Static or molecular SIMS utilizes a very low intensity primary ion beam, and static analyses are typically completed before a single monolayer has

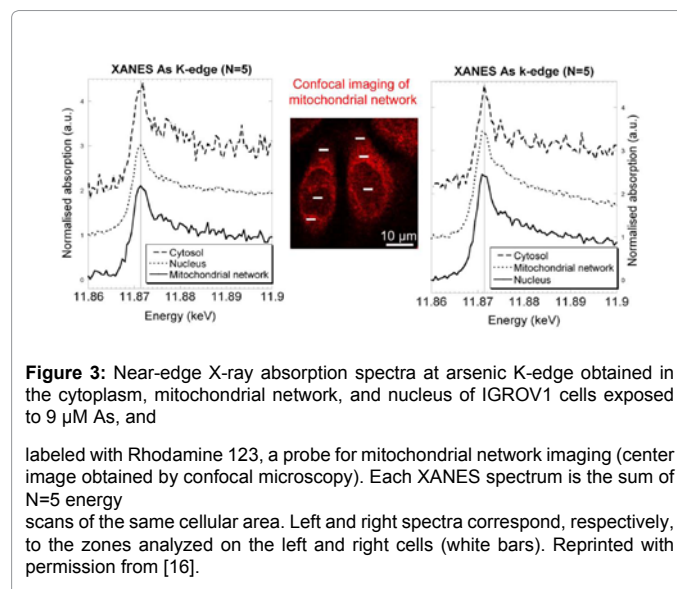
been removed from the surface. Most static analyses are stopped before 1% of the top surface layer has been chemically damaged or eroded; under these conditions, molecular ions and molecular fragment ions characteristic of the chemical structure of the surface are often detected. Thus, static SIMS is best suited for near-surface analysis of molecular composition or chemical structure information, while dynamic SIMS provides the best technique in-depth elemental analysis.

SIMS applications

The use of time-of-flight secondary ion mass spectrometry (ToF-SIMS) as a tool for following the movement of herbicide formulation components into and across plant cuticles has been recently described [18]. This technique provides both high (sub-micron) spatial resolution combined with the chemical specificity associated with organic mass spectrometry. The components studied include the oligomeric ethoxylate surfactants Synperonic A7 and A20 and active ingredient Sulfosate (trimesium glyphosate). The movement of these molecules both separately and when combined in a simple formulation, into the surface of *Prunus laurocerasus* leaves and across the isolated plant cuticle was investigated and clear differences in penetration/diffusion behavior were identified. ToF-SIMS was uniquely able to spatially resolve all the species involved, including the anion and cation components of the active ingredient. In another recent study the uptake visualization of deltamethrin by SIMS and its acute toxicity towards the water flea *D. magna* is described [19]. Bromine from deltamethrin could be visualized by SIMS in tissues of *Daphnia magna* (Figure 4). SIMS was successfully used as a supplemental technique for elucidating the relation between the uptake and localization of deltamethrin and its toxicity to *D. magna*. These results highlight the potential usefulness of SIMS to detect marker elements of xenobiotic compounds within exposed organisms, to compare relative exposure concentrations, and to locate these compounds at their original tissue location.

Conclusion

In conclusion, the aim of this review was to highlight the potential for chemical imaging and speciation micro-analytical methods as complementary tools to other analytical methods to investigate the toxicology of inorganic or organometallic based pesticides. These methods enable to study the intracellular localization of pesticides, determine their chemical speciation, as illustrated in the examples for



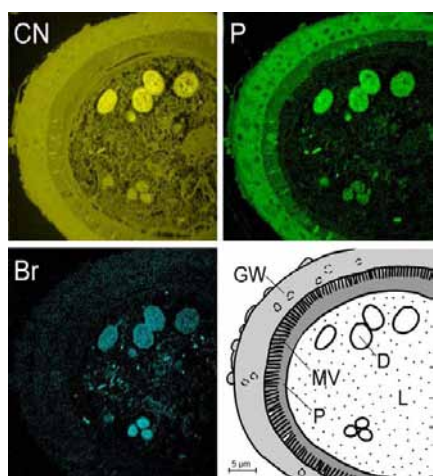


Figure 4: Gut of *D. magna* after 48 h exposure to deltamethrin ($50 \mu\text{g L}^{-1}$) in water. Elements shown are nitrogen (as CN), phosphorus and bromine, as obtained by the NanoSIMS. Brightness indicates higher concentration. Raster size $40 \mu\text{m}$, acquisition time 30 min. D: *Desmodesmus subspicatus* (algae), GW: gut wall, L: lumen with food particles, M: microvilli layer, P: peritrophic membrane. Reprinted with permission from [19].

manganese distribution in neuronal cells and arsenic speciation in ovarian cells. The chemical speciation by XAS is a promising analytical development, especially considering the gain in spatial resolution and detection limits of the last generation XAS microprobes. One can anticipate that the highly sophisticated methods such as SXRF/XAS and SIMS will be more and more used in the near future as the number of facilities available worldwide is increasing. They can offer quite unique information about in situ chemical elements distribution and speciation in cellular organelles.

Acknowledgements

The authors would like to acknowledge the ESRF (European Synchrotron Radiation Facility) for beamtime allocation to study Maneb distribution and Arsenic speciation. We are also sincerely grateful to Murielle Salomé and Sylvain Bohic, respectively from ID21 and ID22 beamlines at ESRF, for their technical support during the experiments.

References

1. Lobinski R, Moulin C, Ortega R (2006) Imaging and speciation of trace elements in biological environment. *Biochimie* 88: 1591-1604.
2. McRae R, Bagchi P, Sumalekshmy S, Fahmi CJ (2009) In situ imaging of metals in cells and tissues. *Chem Rev* 109: 4780-4827.
3. Kim SA, Punshon T, Lanzirotti A, Li L, Alonso JM, et al. (2006) Localization of iron in Arabidopsis seed requires the vacuolar membrane transporter VIT1. *Science* 5803:1295-1298.
4. Roschztardt H, Grillet L, Isaure MP, Conéjéro G, Ortega R, et al. (2011) Plant cell nucleolus as a hot spot for iron. *J Biol Chem* 286: 27863-27866.
5. Goldstein J, Newbury D, Joy D, Lyman C, Echlin P, et al. (2003) In : Scanning Electron Microscopy and X-ray Microanalysis, 3rd Ed., Kluwer Academic/Plenum Publishers, New York.
6. Krause CR (1985) Foliar penetration of a fungicide as detected by scanning electron microscopy and energy dispersive x-ray analysis. *Scan Electron Microsc* 2: 811-815.
7. Hunsche M, Noga G (2009) Spatially resolved quantification of agrochemicals on plant surfaces using energy dispersive X-ray microanalysis. *Pest Manag Sci* 65: 1352-1359.
8. Carmona A, Devès G, Ortega R (2008) Quantitative micro-analysis of metal ions in subcellular compartments of cultured dopaminergic cells by combination of three ion beam techniques. *Anal Bioanal Chem* 390: 1585-1594.
9. Llabador Y, Moretto P (1998) Applications of Nuclear Microprobes in the Life Sciences, World Scientific publishing co, Singapore.
10. Isaure MP, Fraysse A, Devès G, Le Lay P, Fayard B, et al. (2006) Micro-chemical imaging of cesium distribution in Arabidopsis thaliana plant and its interaction with potassium and essential trace elements. *Biochimie* 88: 1583-1590.
11. Mrak T, Simčič J, Pelicon P, Jeran Z, Reis MA, Pinheiro T (2007) Use of micro-PIXE in the study of arsenate uptake in lichens and its influence on element distribution and concentrations. *Nucl Instrum Meth B* 260: 245-253.
12. Laurette J, Larue C, Mariet C, Brisset F, Khodja H, et al. (2012) Influence of uranium speciation on its accumulation and translocation in three plant species: Oilseed rape, sunflower and wheat. *Environ Experim Bot* 77: 96-107.
13. Augustyniak M, Juchimiuk J, Przybyłowicz WJ, Mesjasz-Przybyłowicz J, Babczyńska A, et al. (2006) Zinc-induced DNA damage and the distribution of metals in the brain of grasshoppers by the comet assay and micro-PIXE. *Comp Biochem Physiol C Toxicol Pharmacol* 144: 242-251.
14. Thiruchelvam M, Brockel BJ, Richfield EK, Baggs RB, Cory-Slechta DA (2000) Potentiated and preferential effects of combined paraquat and maneb on nigrostriatal dopamine systems: environmental risk factors for Parkinson's disease? *Brain Res* 873: 225-234.
15. Carmona A, Devès G, Roudeau S, Cloetens P, Bohic S, et al. (2010) Manganese accumulates within Golgi apparatus in dopaminergic cells as revealed by synchrotron X-Ray fluorescence nano-imaging. *ACS Chemical Neurosciences* 1: 194-203.
16. Bacquart T, Devès G, Ortega R (2010) Direct speciation analysis of arsenic in sub-cellular compartments using micro-X-ray absorption spectroscopy. *Environ Res* 110: 413-416.
17. Guerquin-Kern JL, Wu TD, Quintana C, Croisy A (2005) Progress in analytical imaging of the cell by dynamic secondary ion mass spectrometry (SIMS microscopy). *Biochim Biophys Acta* 1724: 228-238.
18. Perkins MC, Bell G, Briggs D, Davies MC, Friedman A, et al. (2008) The application of ToF-SIMS to the analysis of herbicide formulation penetration into and through leaf cuticles. *Colloids Surf B Biointerfaces* 67:1-13.
19. Eybe T, Bohn T, Audinot JN, Udelhoven T, Cauchie HM, et al. (2009) Uptake visualization of deltamethrin by NanoSIMS and acute toxicity to the water flea *Daphnia magna*. *Chemosphere* 76: 134-140.

This article was originally published in a special issue, **Toxicology of Pesticides** handled by Editor(s). Dr. Francisco Sanchez Bayo, Australia; Dr. Richard ORTEGA, France