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Potential Use of Environmental Biological Samples for Retrospective Electron Paramagnetic Resonance Dosimetry of Radiation Accidents

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Abstract: Retrospective dosimetry is one of the most important tools of accident dosimetry for environmental dose estimation when large-scale radiological incidents and nuclear mass-casualty events occur. Electron paramagnetic resonance (EPR) dosimetry is a physical method for the retrospective assessment of absorbed dose based on the measurement of stable radiation-induced radicals in materials. Different from the fast disappearance of radials in aqueous systems, the radials can persist indefinitely in some organized matrices. Therefore, environmental materials contained in creatures from sea or land can be potentially used as environmental dosimeters for a retrospective dose analysis. This study aims to assess the EPR signals of free radicals from environmental biological samples, potentially for the retrospective dose estimation. The evaluated samples involve ox bone, cyclina shell, clam shell, chitin from squid, and human tissue (enamel and fingernail). First, we dehydrate and grind these materials to the powder with different sizes. Subsequently, all materials were irradiated with different doses ranging from 5 Gy to 50 Gy using 6 MV linear accelerator, and EPR spectra of these materials were obtained from the calculation of peak-to-peak amplitudes. The dose-response curve of EPR signals versus irradiated dose for the six materials shows good linearity (R^2~0.99). For the grain-size experiment, the ox bone and tooth with 0.5 mm, the chitin with 0.1 mm, and the others with 1 mm have the strongest signal. For the storage temperature experiment, the optimal temperature of storage is at −20 °C for tooth, fingernail, ox bone, and chitin, at 45 °C for clam shell and cyclina shell where the signal fading is minimal. In conclusion, the developed dose-response curves of the six materials may potentially help a fast, rough retrospective dose reconstruction under the environment when radiation accidents occur.

Keywords: electron paramagnetic resonance; radiation accident; enamel; chitin; cyclina shell; clam shell

1. Introduction

Since the recent Fukushima Daiichi nuclear disaster, concerns about nuclear mass-casualty events has intensified. In the event of a large-scale radiological incident affecting populated areas,

a quantitative assessment of the consequences for human health requires the availability of suitable techniques and procedures for dose reconstruction. Therefore, retrospective assessment of the radiation exposure is vital to the analysis of the radio-epidemiological studies and radiation risk. In addition, retrospective dosimetry is a useful tool for timely assessments of exposures to the general population and the evaluation of individual doses as a basis for the selection of appropriate countermeasures.

For a large-scale radiological accident, the exposure of radiation workers can be found from personal monitors. However, for personnel not equipped with such a device, pursuing an alternative approach is required. Electron paramagnetic resonance (EPR) dosimetry is a method of quantitating the radiation-induced radicals, making it possible to measure the radiation dose absorbed by the irradiated material [1–7]. Owing to this feature, EPR-based dosimetry has been established for many applications such as radiation processing [8], identification of irradiated foods [9,10], radiation oncology [11], evaluation of radiation risk [12], and archeological dating [13].

The tooth enamel is one of the substances established as a suitable dosimeter for individual dose reconstruction following radiation accidents [14–16]. Chandra and Symons [17] have considered the use of human fingernail for measurements of free radical production resulting from radiation damage. However, these materials are only applicable if the human teeth or the fingernail can be readily obtained from accident victims now deceased or from patients in the normal course of dental treatment. The aim of the study is to investigate the use of materials contained in creatures from sea or land potentially as an environmental dosimeter for a retrospective dose analysis in areas and locations where radiation-monitoring measurements were not performed. In this work, EPR technique was applied to irradiated biological samples, investigating the effect of irradiated dose, grain size, and storage temperature with the period following irradiation on the EPR intensity.

2. Materials and Methods

2.1. Preparation of Biological Sample

The biological samples include human tissue (enamel and fingernail), ox bone, cyclina shell, clam shell, and chitin that might be found on or around an irradiated area (Figure 1).

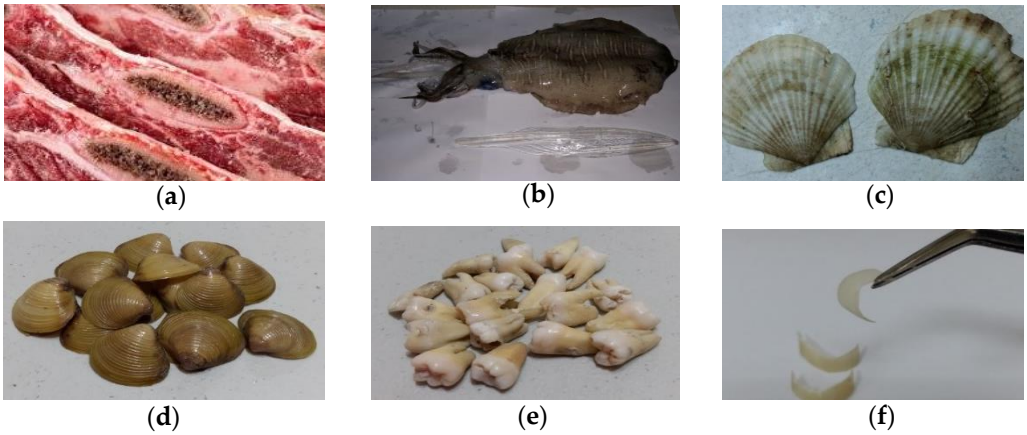

Figure 1. Biological samples of (**a**) ox bone, (**b**) chitin, (**c**) cyclina shell, (**d**) clam shell, (**e**) tooth and (**f**) fingernail.

Fifty male teeth were collected and soaked with 10 M NaOH in test tubes. After the tubes were put into the ultrasonic bath at 50 °C for 72 h, the dentin could be easily separated from the enamel using the disposable blade. All enamel was then placed in an oven at 60 °C for drying. Fingernail samples were collected from about 50 different donors at different times. The fingernail samples were on average 1.5 mm wide and 10 mm long. The ox bone is obtained by removing meat from 1 kg beef short ribs and is placed in the oven until completely dry. The cyclina shells, clam shells and the chitin

extracted from the neritic squid were washed and desiccated at ambient temperature until analyzed. To reduce the mechanical noise caused by the mortar grinding or nail scissors, the powders of enamel, clam shell, and cyclina shell were soaked in 1% acetic acid, while the fingernail and the chitin were soaked in 1% water. Before EPR measurements, the samples need to be dried well to avoid microwave absorption by water [18]. Until the samples were completely dry, each sample was weighed and packed and each sample is 0.2 g.

2.2. EPR Measurement

The samples were measured at National Tsing Hua University using an Elexsys E-580 spectrometer (Bruker, Karlsruhe, Germany) equipped with the super-high-Q resonator cavity (SHQE; scan width constant: 3431–3530, magnetic field modulation amplitude: 0.1 mT, magnetic field modulation frequency: 100 kHz, microwave frequency: 9.75 GHz and microwave power: 15 mW). The Elekta Synergy linear accelerator was used as the radiation source for the biological sample. The peak-to-peak height of EPR signal is obtained by calculating the differences between peaks and troughs of EPR spectra using the amplitude method [19,20]. The EPR signal of each sample contains mechanically induced signal (MIS), native or background signal (BKS) due to the presence of organic radicals, and radiation-induced signals (RIS) [21,22]. Before the exposure experiment, we repeat the measurement of each sample three times and average the results. This averaged value is hereafter subtracted from the measured EPR signal of the irradiated sample to correct the baseline, aiming to remove the MIS and BKS values.

2.3. Experimental Design and Sample Irradiation

The Elekta Synergy linear accelerator is used as radiation source. The dose outputs of the accelerator were calibrated based on the TG-21 protocol under the following conditions: source-to-axis distance (SAD) = 100 cm, field = 10 cm × 10 cm, 1 cGy = 1 Monitor Unit. The expansion of EPR dosimetry into the area of retrospective dose assessment requires a clear understanding of response curve specific to the sample in question. Therefore, the calibration curve (the dose-response curve) of EPR signal intensity versus absorbed dose is established. Each kind of the collected biological samples was split into five sets which were irradiated using a 6 MV photon beam to 5, 10, 20, 40 and 50 Gy to generate the dose-response curve. Note that each set is consisting of three replicates (where the copies with the variation below 5% were selected). In addition, the grain size and the storage temperature were investigated for improving the EPR dosimetry of each biological sample. For grain size test, the biological samples ground with a pestle and mortar were divided into four powder size (1 mm, 0.5 mm, 0.3 mm and 0.1 mm) using the standard sieve. For storage temperature test, the sample was stored at three temperatures of −20 °C, 4 °C and 45 °C with a variable thermostat system.

3. Results

3.1. EPR Spectra

Figure 2 shows the EPR spectra of the six materials received to 5 Gy of photon irradiation. Clam shell, cyclina shell, tooth, and fingernail have a clear and great signal structure, while the EPR intensities of ox bone and chitin are small. As the EPR signal is through the measured material containing substances with unpaired electron spins, the chemical composition of the material plays an important role in the spectral features. The main chemical component of ox bone is $Ca_3(PO_4)_2$; chitin is $(C_8H_{13}O_5N)n$; clam shell and cyclina shell are $CaCO_3$; tooth enamel is $Ca_{10}(PO_4)_6(OH)_2$; fingernail is [S-R-C(NH_2)COOH]n. The ox bone has a complicated and weak spectrum and may be seen as a combination of many different radicals. The similarity of the spectral features in the clam shell and the cyclina shell were observed. The majority of radiation-induced radicals in the clam shell, the cyclina shell, chitin and tooth enamel are carbonate derived [9,15,23] whereas the fingernail may be the sulfonyl radicals [24].

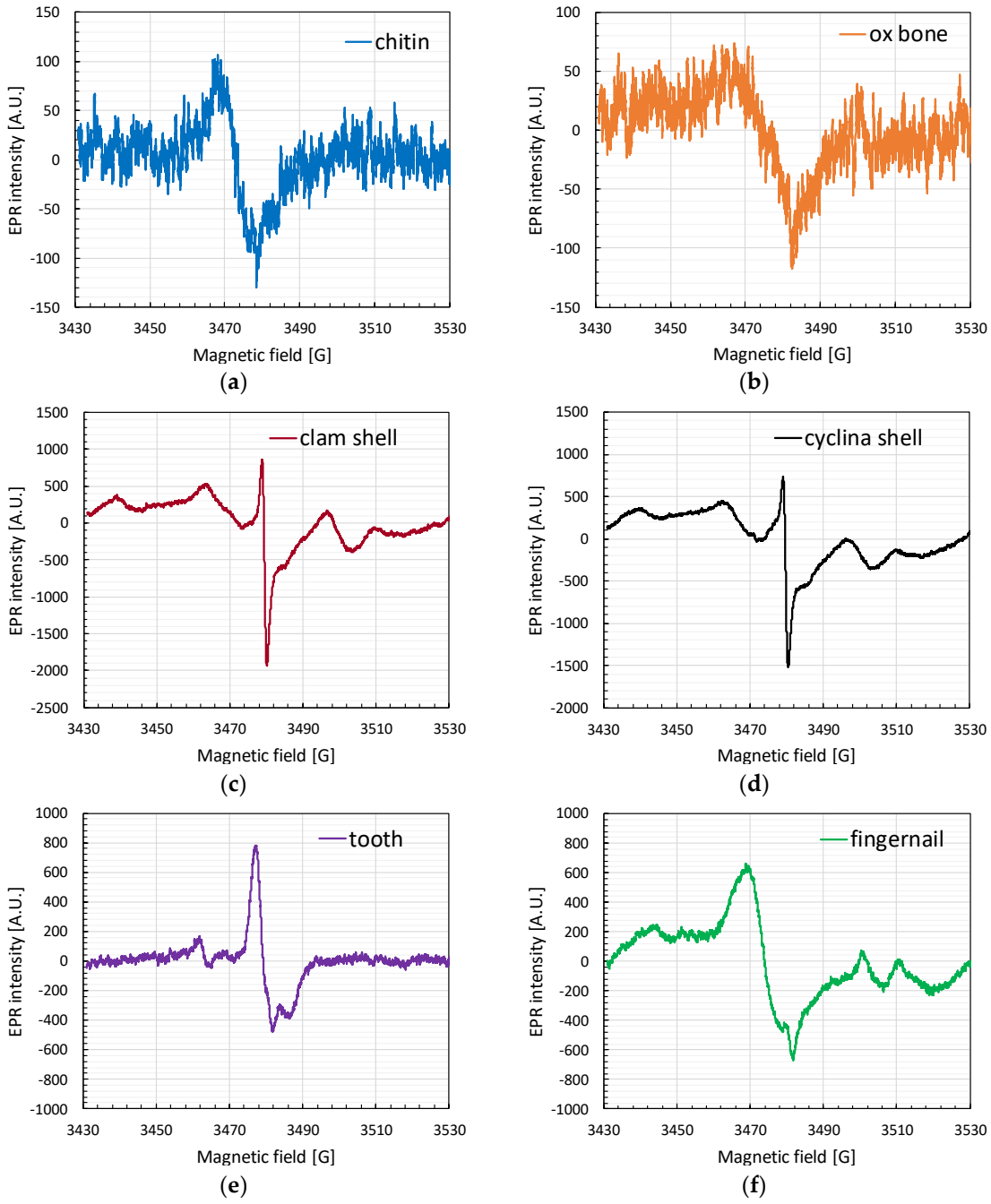

Figure 2. Typical EPR spectra of (**a**) chitin, (**b**) ox bone, (**c**) clam shell, (**d**) cyclina shell, (**e**) tooth and (**f**) fingernail exposed to 5 Gy photon irradiation.

3.2. Dose-Response Curve

The EPR signal intensities of the six materials as a function of absorbed dose shows that the EPR signal intensity increases linearly with absorbed dose ranging from 5 to 50 Gy (shown in Figure 3). When the relationship between EPR signal intensity of stable paramagnetic center and the dose has a linear or almost linear character, the material can be used as a promising dosimeter for quantification of radiation doses. The linear function was fitted to the data using the least square method. All the coefficient of determination, R^2, of the materials can achieve 0.99, indicating a high degree of linearity within this dose range. The slope of the function represents the degree of the radiation sensitivity of the material. Therefore, the clam shell, cyclina shell, tooth enamel, and fingernail have large sensitivity compared to the ox bone and chitin.

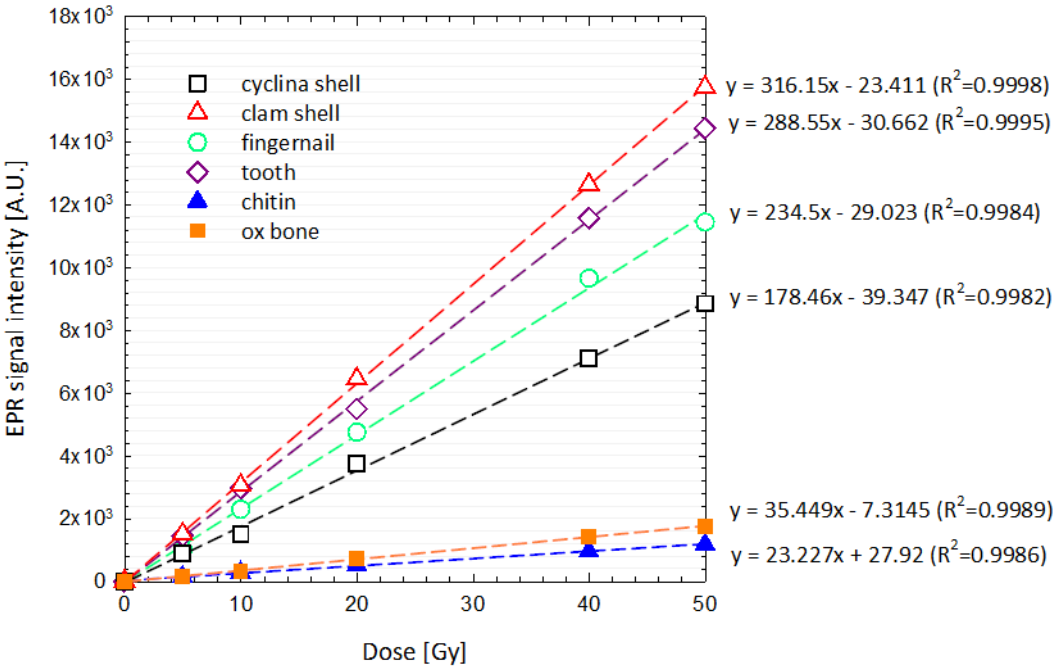

Figure 3. Dose response curve for the six biological samples.

3.3. Grain Size

Grain size between 0.1 and 1 mm is commonly recommended and the maximum sensitivity is varied by the grain size specific to the material [25]. Therefore, the granular effect of the material on the sensitivity of EPR spectra is examined in this section. The grain size of each material is grouped into four sizes of 0.1, 0.3, 0.5 and 1 mm. As shown in Figure 4, the grain size of 1 mm for the clam shell, the cyclina shell and fingernail have the strongest EPR intensity, while the size of ox bone and the tooth is 0.5 mm and chitin is 0.1 mm. Liu et al., (2015) reported that the sensitivity of EPR spectra to exposed dose tends to increase with grain size and reaches saturation [26]. Note that the grain size in 0.1 mm usually shows a different result, which may be due to its lower sensitivity, resulting in a large variation. Aside from the 0.1 mm, the trends of EPR signals as the increase of grain size show upward trends for clam shell, cyclina shell, and fingernail, and stable trends for chitin, ox bone, and tooth, respectively.

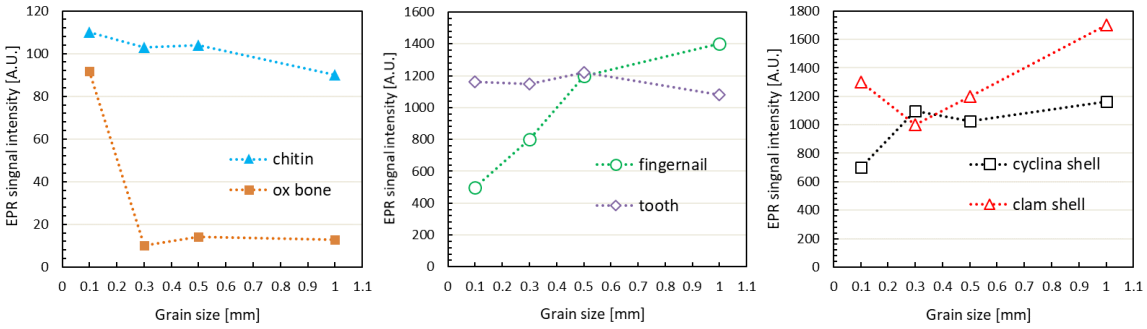

Figure 4. Dose response curve of different grain size for the six biological samples.

3.4. Storage Temperature

Since the loss (fading) of EPR intensity in irradiated samples with the passage of time is affected by the storage of the samples under different temperature [5,27], the temperature dependence of material storage is investigated. The evaluated materials irradiated by 5 Gy photons were grouped. Subsequently, the grouped samples were maintained at three temperatures of −20 °C, 4 °C, and 45 °C, respectively. EPR recordings were made at three-time points of 1 h, 168 h and 336 h at the same

temperature. Representative fading curves for the six materials as a function of storage period up to 14 days are shown in Figure 5. The fading curves show the reduction of 6–25% for the clam shell, 9–15% for the cyclina shell, 2–13% for the tooth, 7–60% for fingernail, 8–38% for chitin, and 9–29% for ox bone. The reduction of fading effect for tooth, fingernail, chitin and ox bone can be considerably reduced at lower temperatures (−20 °C), but the fading effect for clam shell and cyclina shell shows the opposite results. The fading effect for tooth is relatively small, regardless of the storage temperature range [15]. When storage temperature increases, the signal loss of fingernail, chitin, and ox bone with the passage of time was most severe, conforming to the chemical concepts on reaction kinetics and diffusion mobility of radical species. The background signal in the shells is possibly due to trace manganese ions in the calcium lattice. The opposite trends of the shells may lie in the changes of Mn2+ background signal during the sample preparation between measuring the background EPR signals and measuring the post-exposure EPR signals [28]. It was clearly seen that the fading effect of EPR signal highly depends on the storage temperature and period after irradiation.

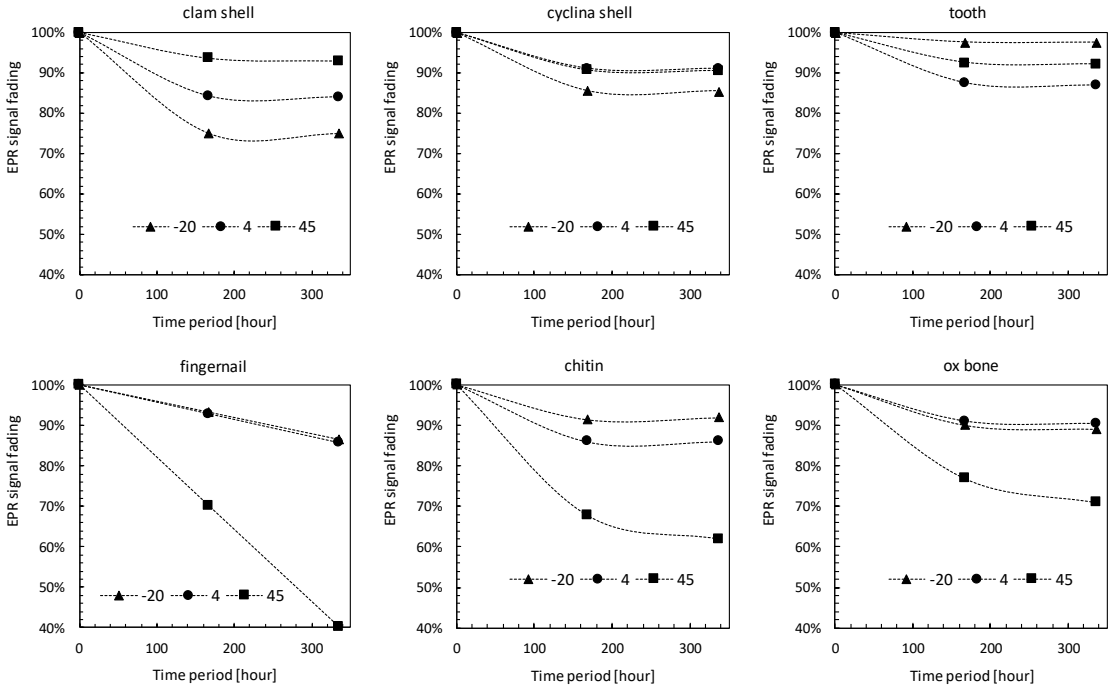

Figure 5. EPR signal fading for the six biological samples stored at −20, 4 and 45 °C.

4. Discussion and Conclusions

Herein, the present work reveals that EPR technique can be successfully applied to the identification of irradiated clam shell, cyclina shell, fingernail, tooth, chitin and ox bone. All the biological samples show a good linearity of the measured EPR signals against the dose, despite the fact that the chitin and ox bone have lower radiation sensitivity. The optimized grain size for maximizing the signal is 1 mm for the clam shell, cyclina shell, and fingernail, 0.5 mm for ox bone and tooth and 0.1 mm for chitin. The fading of EPR signal with the passage of time can be considerably alleviated by control the storage temperature at −20 °C for tooth, fingernail, ox bone and chitin and at 45 °C for clam shell and cyclina shell. These results preliminarily support that the biological samples have the potential to become an environmental dosimeter for retrospectively giving a fast rough estimate of dose level under the environment when radiation accidents occur.

On the other hand, our results were obtained by sample preparation of the biological materials before radiation; this can be considered as one of main uncertainties of the presented analysis. In an actual event, real tissue will have been irradiated in situ before the samples are collected and analyzed, but the biological samples in the study are prepared before irradiation. The procedure for

sample preparation may affect the nature of the materials (such as water content) and its behavior to radiation, leading to any effect of ionizing radiation to be undependable as a surrogate for retrospective studies [29,30]. This may need further calibration work to overcome the issues. Nevertheless, our study has shown the potential use of the six materials as environmental dosimeters and justify continued efforts to develop the method of environmental dose estimation.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Liu, C.Y.; Lai, L.H.; Wang, C.F.; Chuang, K.S.; Lu, C.C.; Lin, J.P.; Lin, H.H. Establishment of conversion coefficient of whole body effective dose by human tissue of electron paramagnetic resonance (EPR). *Radiat. Phys. Chem.* **2019**, *155*, 82–88. [CrossRef]
2. Desrosiers, M.F.; Puhl, J.M. Absorbed-dose/dose-rate dependence studies for the alanine-EPR dosimetry system. *Radiat. Phys. Chem.* **2009**, *78*, 461–463. [CrossRef]
3. Ikeya, M.; Miyajima, J.; Okajima, S. ESR dosimetry for atomic bomb survivors using shell buttons and tooth enamel. *Jpn. J. Appl. Phys.* **1984**, *23*, L697. [CrossRef]
4. Sudprasert, W.; Monthonwattana, S.; Vitittheeranon, A. Identification of irradiated rice noodles by electron spin resonance spectroscopy. *Radiat. Meas.* **2012**, *47*, 640–643. [CrossRef]
5. Trompier, F.; Kornak, L.; Calas, C.; Romanyukha, A.; LeBlanc, B.; Mitchell, C.A.; Swartz, H.M.; Clairand, I. Protocol for emergency EPR dosimetry in fingernails. *Radiat. Meas.* **2007**, *42*, 1085–1088. [CrossRef]
6. Yordanov, N.D.; Aleksieva, K. Preparation and applicability of fresh fruit samples for the identification of radiation treatment by EPR. *Radiat. Phys. Chem.* **2009**, *78*, 213–216. [CrossRef]
7. Yordanov, N.D.; Mladenova, B. EPR studies on gamma-irradiated snails hard tissues. *Radiat. Phys. Chem.* **2001**, *60*, 191–193. [CrossRef]
8. Soliman, Y.; Ali, L.I.; Moustafa, H.; Tadros, S.M. EPR dosimetric properties of 2-methylalanine pellet for radiation processing application. *Radiat. Phys. Chem.* **2014**, *102*, 11–15. [CrossRef]
9. Desrosiers, M.F. gamma.Irradiated seafoods: Identification and dosimetry by electron paramagnetic resonance spectroscopy. *J. Agric. Food Chem.* **1989**, *37*, 96–100. [CrossRef]
10. Stachowicz, W.; Burlinska, G.; Michalik, J. EPR detection of foods preserved with ionizing radiation. *Radiat. Phys. Chem.* **1998**, *52*, 157–160. [CrossRef]
11. Anton, M. Uncertainties in alanine/ESR dosimetry at the Physikalisch-Technische Bundesanstalt. *Phys. Med. Biol.* **2006**, *51*, 5419–5440. [CrossRef] [PubMed]
12. Jacob, P.; Bailiff, I.K.; Balonov, M.A.; Bauchinger, M.; Bouville, A.; Haskell, E.; Nakamura, N.; Romanyukha, A. Retrospective assessment of exposures to ionising radiation: Abstract. *ICRU* **2002**, *2*, 9. [CrossRef]
13. Cano, N.F.; Munita, C.S.; Watanabe, S.; Barbosa, R.F.; Chubaci, J.F.; Tatumi, S.H.; Neves, E.G. OSL and EPR dating of pottery from the archaeological sites in Amazon Valley, Brazil. *Quat. Int.* **2014**, *352*, 176–180. [CrossRef]
14. Egersdörfer, S.; Wieser, A.; Müller, A. Tooth enamel as a detector material for retrospective EPR dosimetry. *Appl. Radiat. Isot.* **1996**, *47*, 1299–1303. [CrossRef]
15. Fattibene, P.; Callens, F. EPR dosimetry with tooth enamel: A review. *Appl. Radiat. Isot.* **2010**, *68*, 2033–2116. [CrossRef]
16. Ivannikov, A.I.; Sanin, D.; Nalapko, M.; Skvortsov, V.F.; Stepanenko, V.F.; Tsyb, A.F.; Trompier, F.; Zhumadilov, K.; Hoshi, M. Dental enamel EPR dosimetry: Comparative testing of the spectra processing methods for determination of radiation-induced signal amplitude. *Health Phys.* **2010**, *98*, 345–351. [CrossRef]

17. Chandra, H.; Symons, M.C. Sulphur radicals formed by cutting α-keratin. *Nature* **1987**, *328*, 833–834. [CrossRef]
18. Sholom, S.; McKeever, S. Stability of X-band EPR signals from fingernails under vacuum storage. *Radiat. Phys. Chem.* **2017**, *141*, 78–87. [CrossRef]
19. Chumak, V.; Bailiff, I.; Baran, N.; Bugai, A.; Dubovsky, S.; Fedosov, I.; Finin, V.; Haskell, E.; Hayes, R.; Ivannikov, A.; et al. The first international intercomparison of EPR-dosimetry with teeth: First results. *Appl. Radiat. Isot.* **1996**, *47*, 1281–1286. [CrossRef]
20. Haskell, E.H.; Hayes, R.B.; Kenner, G.H.; Wieser, A.; Aragno, D.; Fattibene, P.; Onori, S. Achievable precision and accuracy in EPR dosimetry of tooth enamel. *Radiat. Prot. Dosim.* **1999**, *84*, 527–535. [CrossRef]
21. Swartz, H.M.; Flood, A.B.; Williams, B.B.; Dong, R.; Swarts, S.G.; He, X.; Grinberg, O.; Sidabras, J.; Demidenko, E.; Gui, J.; et al. Electron paramagnetic resonance dosimetry for a large-scale radiation incident. *Health Phys.* **2012**, *103*, 255–267. [CrossRef] [PubMed]
22. Symons, M.C.R.; Chandra, H.; Wyatt, J.L. Electron paramagnetic resonance spectra of irradiated finger-nails: A possible measure of accidental exposure. *Radiat. Prot. Dosim.* **1995**, *58*, 11–15.
23. Sadło, J.; Michalik, J.; Stachowicz, W.; Strzelczak, G.; Dziedzic-Gocławska, A.; Ostrowski, K. EPR study on biominerals as materials for retrospective dosimetry. *Nukleonika* **2006**, *51*, 95–100.
24. Trompier, F.; Romanyukha, A.; Reyes, R.; Vezin, H.; Queinnec, F.; Gourier, D. State of the art in nail dosimetry: Free radicals identification and reaction mechanisms. *Biophysik* **2014**, *53*, 291–303. [CrossRef]
25. Hong, D.; Lee, K. Grain size effect of tooth enamel to electron paramagnetic resonance spectrum. *Nucl. Sci. Technol.* **2004**, *41*, 200–202.
26. Liu, C.R.; Yin, G.M.; Han, F. Effects of grain size on quartz ESR dating of Ti–Li center in fluvial and lacustrine sediments. *Quat. Geochronol.* **2015**, *30*, 513–518. [CrossRef]
27. Romanyukha, A.; Trompier, F.; LeBlanc, B.; Calas, C.; Clairand, I.; Mitchell, C.A.; Smirniotopoulos, J.G.; Swartz, H.M. EPR dosimetry in chemically treated fingernails. *Radiat. Meas.* **2007**, *42*, 1110–1113. [CrossRef] [PubMed]
28. Blanchard, S.C.; Chasteen, N.D. Electron paramagnetic resonance spectrum of a sea shell. Mytilus edulis. *J. Phys. Chem.* **1976**, *80*, 1362–1367. [CrossRef]
29. Viscomi, D.; Fattibene, P. Radiation-induced signals analysed by EPR spectrometry applied to fortuitous dosimetry. *Annali dell'Istituto Superiore di Sanità* **2009**, *45*, 287–296.
30. Bougai, A.; Brik, A.; Chumak, V.; Desrosiers, M.; Dubovski, S.; Fattibene, P.; Romanyukha, A. *Use of Electron Paramagnetic Resonance Dosimetry with Tooth Enamel for Retrospective Dose Assessment*; TECDOC-1331; International Atomic Energy Agency: Vienna, Austria, 2002.