Article

Monitoring Lead (Pb) Pollution and Identifying Pb Pollution Sources in Japan Using Stable Pb Isotope Analysis with Kidneys of Wild Rats

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Abstract: Although Japan has been considered to have little lead (Pb) pollution in modern times, the actual pollution situation is unclear. The present study aims to investigate the extent of Pb pollution and to identify the pollution sources in Japan using stable Pb isotope analysis with kidneys of wild rats. Wild brown (*Rattus norvegicus*, n = 43) and black (*R. rattus*, n = 98) rats were trapped from various sites in Japan. Mean Pb concentrations in the kidneys of rats from Okinawa (15.58 mg/kg, dry weight), Aichi (10.83), Niigata (10.62), Fukuoka (8.09), Ibaraki (5.06), Kyoto (4.58), Osaka (4.57), Kanagawa (3.42), and Tokyo (3.40) were above the threshold (2.50) for histological kidney changes. Similarly, compared with the previous report, it was regarded that even structural and functional kidney damage as well as neurotoxicity have spread among rats in Japan. Additionally, the possibility of human exposure to a high level of Pb was assumed. In regard to stable Pb isotope analysis, distinctive values of stable Pb isotope ratios (Pb-IRs) were detected in some kidney samples with Pb levels above 5.0 mg/kg. This result indicated that composite factors are involved in Pb pollution. However, the identification of a concrete pollution source has not been accomplished due to limited differences among previously reported values of Pb isotope composition in circulating Pb products. Namely, the current study established the limit of Pb isotope analysis for source identification. Further detailed research about monitoring Pb pollution in Japan and the demonstration of a novel method to identify Pb sources are needed.

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1. Introduction

Among toxic metals, lead (Pb) exhibits a particularly elevated anthropogenic enrichment factor [1]. Pb moves into the environment during use (including batteries and paint), recycling, and disposal of Pb compounds; metal production processes (mining and smelting activities); combustion of fossil fuels (coal, former use of leaded gasoline); and sewage sludge applications [2–4]. Although leaded gasoline has been strictly regulated all over the world, the use of Pb-based products has increased greatly since the Industrial Revolution. As a result, Pb pollution does not only occur locally but also worldwide due to its volatile character [5]. Additionally, Pb is a proven non-essential and toxic metal for humans and animals [6–9]. For humans, Pb is known to be neurotoxic, especially to children because of its ability to compete with calcium (Ca^{2+}) in nerve functioning [10]. Moreover, it has been thoroughly studied that Pb poisoning progresses from biochemical and subclinical abnormalities, such as gastrointestinal problems, stunted growth, and cognition problems, to death [9]. These types of Pb poisoning cases have been reported in humans worldwide. For instance, more than 160 people, mainly children under the age of five, died in the Pb poisoning disaster in Zamfara, Nigeria [11].

In addition to considering the total levels and chemical/mineralogy composition of Pb, the contribution of Pb to the environment from multiple sources should be accurately determined. Among the natural abundance of four stable isotopes of Pb, i.e., ^{208}Pb, ^{207}Pb, ^{206}Pb, and ^{204}Pb, only ^{204}Pb is not radiogenic. The abundance of ^{204}Pb in the Earth's crust has remained since the Earth solidified. On the other hand, ^{208}Pb, ^{207}Pb, and ^{206}Pb are radiogenic isotopes, and are the products of the radioactive decay of ^{232}Th, ^{235}U, and ^{238}U, respectively. The abundance of Pb isotopes in a sample, therefore, strictly depends on the concentrations of primordial Pb, uranium (U), and thorium (Th) isotopes, and the length of their decay processes [12]. In addition, the isotopic Pb composition is not significantly affected by physico-chemical fractionation processes [13,14]. Hence, identifying the Pb pollution source using stable Pb isotope ratios (Pb-IRs) has been widely carried out in many fields of environmental research, including in air and aerosols [15], soil [16], birds [17], and marine mammals [18].

It has been reported that people in pre-industrialized Japan were highly contaminated with Pb of domestic origin [19]. By contrast, after industrialization, which was accompanied by the import of foreign Pb-containing materials including leaded gasoline, pollution with foreign Pb, Pb-IRs of which differ from domestic materials, occurred [19,20]. Recent studies focused on the contemporary Pb level in Japanese human samples reported relatively low concentrations of Pb in cord blood [21], children's blood [22], and women's hair [20]. However, the maximum weekly intake per kg body weight (bw) for a five-year-old Japanese child was surprisingly revealed to be 26 μg/kg bw [23]. This value is higher compared to the provisional tolerable weekly intake (PTWI) for Pb of 25 μg/kg bw, which is recommended by the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee in Food Additives (JECFA) [24]. Furthermore, high levels of Pb were previously confirmed in montane soil [25], public playground soil [26], and road-side dust and sediment [27]. Besides, Bellis et al. [28] provided evidence of the long-range transport of Pb from continental Asia to Japan. For these reasons, environmental monitoring of Pb pollution is necessary to prevent risking human health in Japan.

To evaluate the biological effects of Pb in detail, a model based on a living organism is needed. Inherent and external factors, such as size, sensitivity, physiological characteristics, position in the food chain, migration, abundance, and ability to propagate in captivity, were widely regarded as criteria for a good sentinel [29]. From these standpoints, the wild rat is large enough as a sentinel, and they actually have been used as mammalian sentinels for terrestrial metal pollution [30–33]. Besides, a

previous report revealed that Pb-IRs in the rat kidney comparatively accurately reflect those of the Pb source, although biological fractionation of Pb isotopes was speculated [34]. Thus, it is likely that a Pb pollution source could be identified using Pb isotope analysis of rat kidneys as mentioned above. As a consequence, the current study aims to elucidate the extent of Pb contamination and to identify Pb sources in Japan using kidneys of wild rats. To the best of our knowledge, this is the first study that focuses on Pb-IRs in wild rats.

2. Materials and Methods

2.1. Sampling of Wild Rats

Wild rats, including brown (*Rattus norvegicus*, n = 43) and black rats (*R. rattus*, n = 98) were collected from various sampling sites in Japan (Figure 1) from July 2004 to November 2013 using gauze cage traps with food. Rat species were morphologically identified. Kidney samples were collected from both *R. norvegicus* and *R. rattus*, and kept at −20 °C until further use.

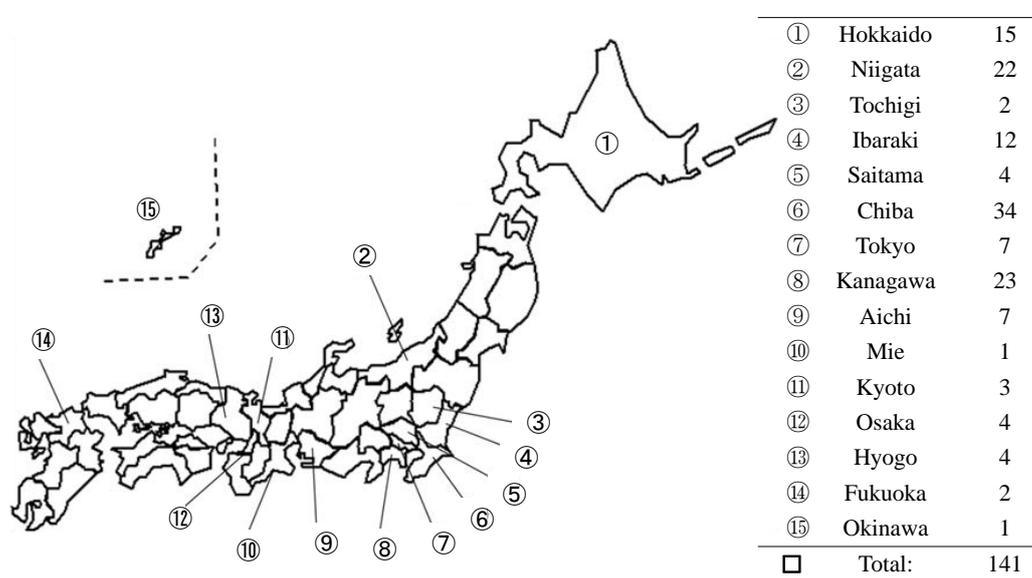|		
①	Hokkaido	15
②	Niigata	22
③	Tochigi	2
④	Ibaraki	12
⑤	Saitama	4
⑥	Chiba	34
⑦	Tokyo	7
⑧	Kanagawa	23
⑨	Aichi	7
⑩	Mie	1
⑪	Kyoto	3
⑫	Osaka	4
⑬	Hyogo	4
⑭	Fukuoka	2
⑮	Okinawa	1
	Total:	141

Figure 1. Distribution and number of rat samples by region.

2.2. Sample Preparations and Extraction

All laboratory materials and instruments used in the current study were washed with 2% nitric acid (HNO$_3$, atomic absorption spectrometry grade, Kanto Chemical Corp., Tokyo, Japan) and rinsed at least twice with distilled water. For acid digestion, a dry thermal unit was used following methodology modified from Nakayama et al. [33]. Kidney samples (1.0 g or available amount) were dried in heat resistant glass tubes for 48 h. The dry weight was calculated, and the dried samples were then digested in 5 mL of 60% HNO$_3$ for 48 h in a dry thermal unit. The temperature was increased gradually to 140 °C, and after complete digestion, the acid was evaporated at 160 °C to reduce the volume to 0.5 mL. The cooled samples were transferred into 15 mL plastic tubes, followed by dilution to 10 mL with 2% HNO$_3$. The resulting samples were stored at room temperature in preparation for spectrophotometry analysis. Replicate blanks and the certified reference material DOLT-4 (dogfish liver, National Research Council of Canada: Ottawa, ON, Canada) were used for method validation and quality control. Replicate analysis of these reference materials showed good accuracy, with recovery rates of 90% ± 3.5%.

2.3. Analysis of Pb Concentration

The Pb concentration was determined with a flame/flame-less atomic absorption spectrophotometer (AAS, Z-2010, Hitachi High-Technologies Corporation, Tokyo, Japan) equipped with a Zeeman graphite furnace or an inductively coupled plasma–mass spectrometer (ICP-MS: 7700 series, Agilent Technologies, Tokyo, Japan). The instrument was calibrated using standard stock solutions (Kanto Chemical Corp.). A flame-less method with argon gas was used for measuring the Pb concentration.

2.4. Analysis of Pb-IRs

Analyses of the Pb-IRs (^{208}Pb/^{206}Pb and ^{207}Pb/^{206}Pb) were conducted using ICP-MS, according Nakata et al. [35]. For precise analysis, the extracted solutions from the kidney samples were diluted so that Pb concentration in the solutions became less than 40 µg/L. In order to correct for mass bias and dead-time effects, standard reference material (SRM) 981 (National Institute of Standards and Technology; NIST, Gaithersburg, MD, USA) was measured at every 10 samples. During the analytical procedure, the following isotopes were measured: ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb. However, only the ^{208}Pb/^{206}Pb and ^{207}Pb/^{206}Pb ratios are discussed in this study, as these ratios have been commonly interpreted in previous research [36]. The ratios of the samples were corrected at every 10 samples using the average value of each isotope ratio obtained from the SRM 981 measurement. The standard error for the ^{208}Pb/^{206}Pb and ^{207}Pb/^{206}Pb measurements was <0.4% of relative standard deviation (RSD). There was no metal contamination through the analytical procedures using the reagent (digestion) blank measurement.

2.5. Statistical Analysis

The Pb concentration data were log-transformed to stabilize the variances. All statistical analyses were carried out using JMP 12 (SAS Institute, Cary, NC, USA). Significant difference in the Pb concentrations of the kidney between *R. norvegicus* and *R. rattus* were evaluated by the Student's *t*-test. Significant differences in the Pb concentrations and Pb-IRs of the kidney among various regions were analyzed by the Tukey-Kramer test, except for Okinawa, Fukuoka, Tochigi, and Mie of which sample sizes are small. All the statistical analyses were performed at a significance level of 0.05 ($\alpha = 0.05$, $p < 0.05$).

3. Results

3.1. Pb Concentrations in Kidneys of Wild Rats

The Pb concentrations in the kidneys of wild rats were determined (Table 1). No significant difference was observed in the pattern of Pb accumulation between *R. norvegicus* and *R. rattus*. In addition, it has been reported that the accumulation pattern of metals in *R. norvegicus* and *R. rattus* shows limited differences [37]. Therefore, the rats were not distinguished in the present study.

As shown in Table 1, rats from Aichi accumulated significantly higher concentrations of Pb in their kidneys compared with rats from Chiba and Hokkaido. Similarly, a significantly higher level of Pb was observed in the rat kidneys from Niigata than those from Kanagawa, Chiba, and Hokkaido. Additionally, the Pb concentrations in the kidneys of the rats from Ibaraki were significantly higher compared with those of the rats from Hokkaido. A high variability in the Pb level was observed in the kidney samples from every location (Table 1).

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Table 1. Pb concentration (mg/kg, dry weight) in kidneys of wild rats from various regions in Japan.

Regions	No. of Samples	Mean ± SD	Median	Range
Okinawa	total (n = 1)	15.58	NA	NA
	RN (n = 0)	-	-	-
	RR (n = 1)	15.58	NA	NA
Aichi [a,b]	total (n = 7)	10.83 ± 12.58	NA	NA
	RN (n = 0)	-	-	-
	RR (n = 7)	10.83 ± 12.58	NA	NA
Niigata [a]	total (n = 22)	10.62 ± 11.08	5.51	0.40–42.63
	RN (n = 2)	0.79, 30.70	NA	NA
	RR (n = 20)	10.95 ± 11.38	5.51	0.40–42.63
Fukuoka	total (n = 2)	2.50, 13.68	NA	NA
	RN (n = 0)	-	-	-
	RR (n = 2)	2.50, 13.68	NA	NA
Ibaraki [a,b,c]	total (n = 12)	5.06 ± 6.68	1.90	1.07–22.80
	RN (n = 0)	-	-	-
	RR (n = 12)	5.06 ± 6.68	1.90	1.07–22.80
Kyoto [a,b,c,d]	total (n = 3)	4.58 ± 3.33	4.34	1.37 ± 8.02
	RN (n = 2)	1.37, 4.34	NA	NA
	RR (n = 1)	8.02	NA	NA
Osaka [a,b,c,d]	total (n = 4)	4.57 ± 4.03	3.83	0.49–10.13
	RN (n = 2)	3.71, 10.13	NA	NA
	RR (n = 2)	0.49, 3.96	NA	NA
Kanagawa [b,c,d]	total (n = 23)	3.42 ± 5.29	1.03	0.26–20.56
	RN (n = 19)	3.04 ± 5.64	0.86	0.26–20.56
	RR (n = 4)	4.09 ± 2.73	4.55	0.49–6.78
Tokyo [a,b,c,d]	total (n = 7)	3.40 ± 3.81	1.86	1.09–11.78
	RN (n = 3)	5.60 ± 5.53	3.93	1.09–11.78
	RR (n = 4)	1.75 ± 0.27	1.75	1.44–2.05
Tochigi	total (n = 2)	0.98, 3.81	NA	NA
	RN (n = 0)	-	-	-
	RR (n = 2)	0.98, 3.81	NA	NA
Hyogo [a,b,c,d]	total (n = 4)	2.30 ± 2.81	1.07	0.58–6.50
	RN (n = 3)	2.78 ± 3.24	1.26	0.57–6.50
	RR (n = 1)	0.87	NA	NA
Saitama [a,b,c,d]	total (n = 4)	2.16 ± 1.47	2.43	0.23–3.55
	RN (n = 1)	0.23	NA	NA
	RR (n = 3)	2.80 ± 0.87	3.02	1.84, 3.55
Chiba [c,d]	total (n = 34)	1.88 ± 2.57	1.11	0.02–10.26
	RN (n = 7)	3.66 ± 3.02	1.83	1.16–8.32
	RR (n = 27)	1.42 ± 2.28	0.59	0.02–10.26
Hokkaido [d]	total (n = 15)	0.69–0.41	0.65	0.09–1.59
	RN (n = 3)	0.48 ± 0.32	0.65	0.11–0.68
	RR (n = 12)	0.74 ± 0.42	0.69	0.09–1.59
Mie	total (n = 1)	0.09	NA	NA
	RN (n = 1)	0.09	NA	NA
	RR (n = 0)	-	-	-

RN = *R. norvegicus*. RR = *R. rattus*. Different lowercase letters (a, b, c, and d) indicate a significant difference among regions. No significance test was conducted for Okinawa, Fukuoka, Tochigi, and Mie, due to small sample size. NA means not analyzed due to small sample size.

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3.2. Pb-IRs in the Kidneys of Wild Rats

Geographic trends of the kidney Pb-IRs are shown in Figure 2, and the trends classified by the Pb level in the kidney (Pb < 2.5 mg/kg (dry weight, Figure 3a), 2.5 mg/kg ≤ Pb < 5.0 mg/kg (Figure 3b), 5.0 mg/kg ≤ Pb < 15.0 mg/kg (Figure 3c), 15.0 mg/kg ≤ Pb (Figure 3d)) are shown in Figure 3. These separation values of 2.5 mg/kg and 15.0 mg/kg for the classification were chosen following the previous reported threshold values for the detection of histological kidney changes [30] and structural as well as functional kidney damage [38], respectively. Another separation value of 5.0 mg/kg was set because a different tendency was observed at the Pb concentration ≥5.0 mg/kg as described below. Mean ± SD, median, and minimum-maximum values of the Pb-IRs are shown in Table 2.

Table 2. Pb-IRs in kidneys of wild rats from various regions in Japan.

Regions (No. of Samples)	$^{208}Pb/^{206}Pb$			$^{207}Pb/^{206}Pb$		
	Mean ± SD	Median	Range	Mean ± SD	Median	Range
Okinawa (n = 1)	2.109	NA	NA	0.867	NA	NA
Aichi (n = 7) [a,b]	2.120 ± 0.011	2.120	2.102–2.133	0.871 ± 0.006	0.872	0.861–0.876
Niigata (n = 22) [b]	2.105 ± 0.013	2.106	2.079–2.126	0.862 ± 0.005	0.862	0.849–0.871
Fukuoka (n = 2)	2.093, 2.110	NA	NA	0.859, 0.865	NA	NA
Ibaraki (n = 12) [a,b]	2.113 ± 0.026	2.116	2.076–2.175	0.866 ± 0.020	0.866	0.843–0.922
Kyoto (n = 3) [a,b]	2.122 ± 0.004	2.120	2.119–2.126	0.871 ± 0.002	0.872	0.870–0.873
Osaka (n = 4) [a,b]	2.109 ± 0.012	2.107	2.098–2.124	0.863 ± 0.009	0.860	0.855–0.876
Kanagawa (n = 23) [a,b]	2.105 ± 0.018	2.104	2.056–2.138	0.865 ± 0.012	0.866	0.825–0.884
Tokyo (n = 7) [a,b]	2.119 ± 0.009	2.119	2.104–2.132	0.873 ± 0.006	0.873	0.863–0.880
Tochigi (n = 2)	2.098, 2.117	NA	NA	0.859, 0.872	NA	NA
Hyogo (n = 4) [a,b]	2.107 ± 0.011	2.106	2.096–2.120	0.865 ± 0.006	0.865	0.858–0.872
Saitama (n = 4) [a,b]	2.114 ± 0.004	2.114	2.109–2.119	0.866 ± 0.003	0.867	0.861–0.868
Chiba (n = 34) [a]	2.120 ± 0.011	2.121	2.101–2.162	0.874 ± 0.007	0.873	0.865–0.909
Hokkaido (n = 15) [a,b]	2.110 ± 0.009	2.109	2.098–2.129	0.867 ± 0.005	0.867	0.858–0.875
Mie (n = 1)	2.104	NA	NA	0.861	NA	NA

Different lowercase letters (a and b) indicate a significant difference in both $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$ among regions. No significance test was conducted for Okinawa, Fukuoka, Tochigi, and Mie, due to small sample size. NA means not analyzed due to small sample size.
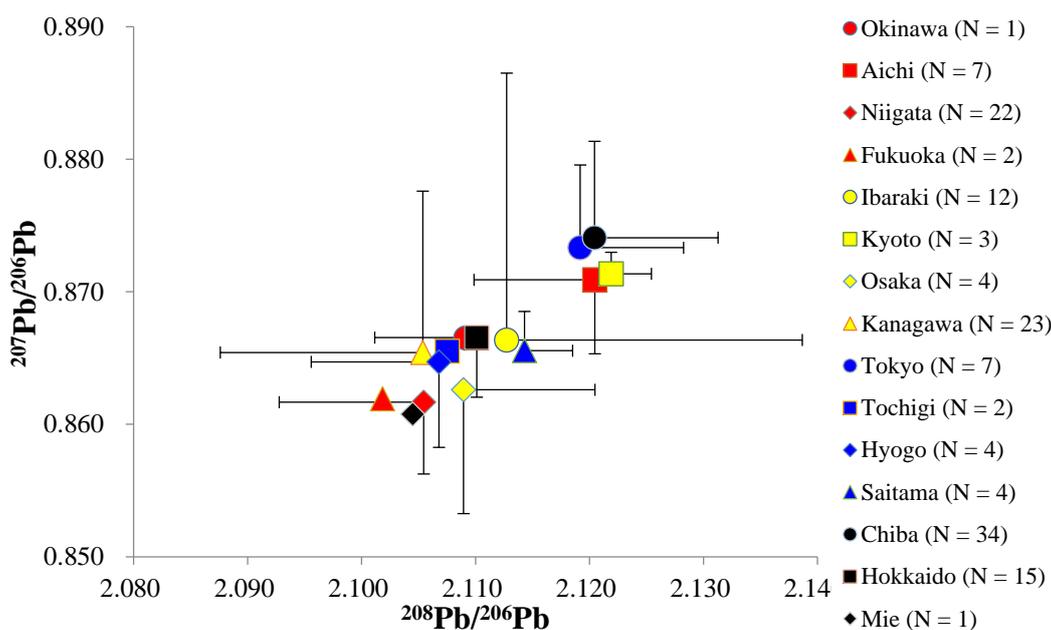

Figure 2. Pb-IRs ($^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$) in kidneys of wild rats from various regions in Japan.
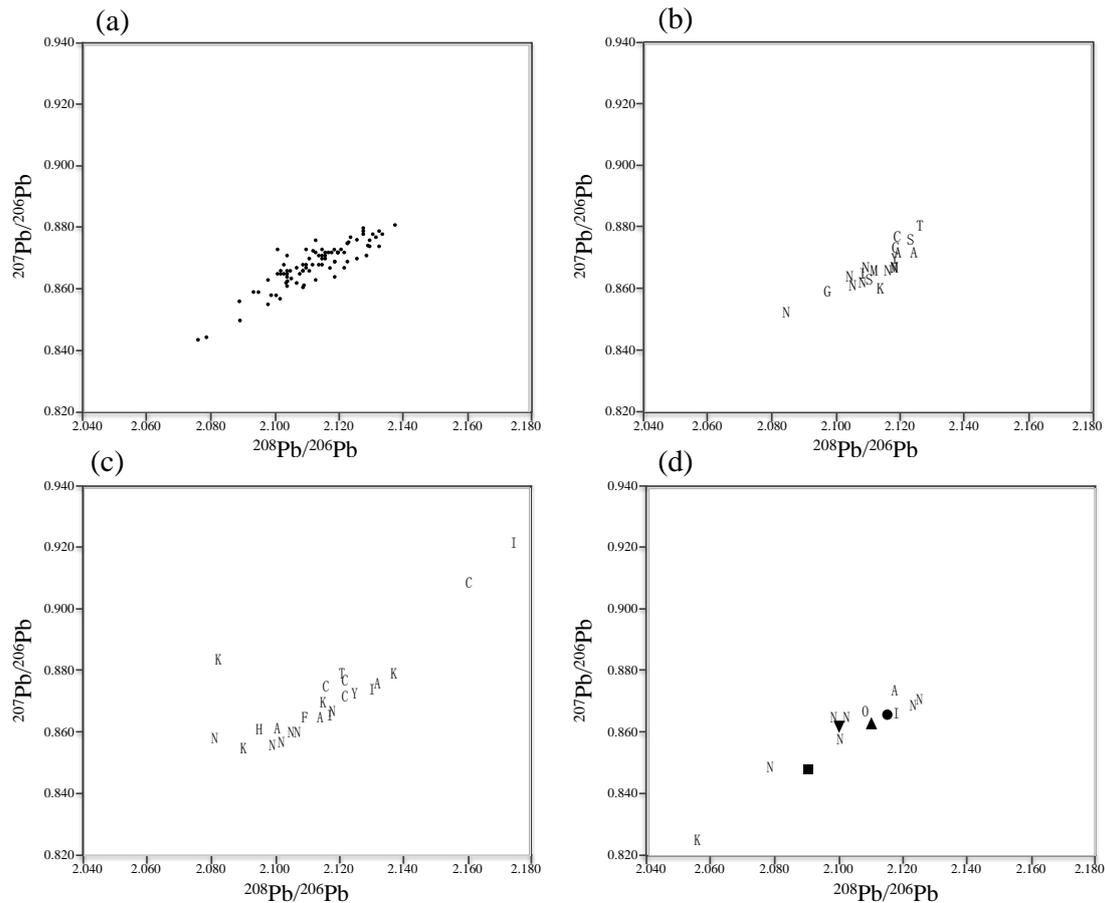
Figure 3. Pb-IRs in kidneys of wild rat from various regions in Japan. Pb level in kidney < 2.5 mg/kg (**a**); 2.5 ≤ Pb level in kidney < 5.0 (**b**); 5.0 ≤ Pb level in kidney < 15.0 (**c**); 15.0 ≤ Pb level in kidney (**d**). A = Aichi, C = Chiba, F = Fukuoka, G = Tochigi, H = Hyogo, I = Ibaraki, K = Kanagawa, M = Saitama, N = Niigata, O = Okinawa, S = Osaka, T = Tokyo, Y = Kyoto. Circle = solder circulated in Japan [39], square = paint [39], triangle = battery [40], inverted triangle = airborne particulate matter [39]. Figure 3a was shown with simple dots due to too many individuals.

The ratios of both $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$ in the kidneys of the rats from Chiba were significantly higher compared with those ratios in the kidneys of the rats from Niigata; however, the mean values of Pb-IRs in the kidneys of rats from each region generally converged closely at around approximately 2.100 to 2.120 ($^{208}Pb/^{206}Pb$) and 0.860 to 0.875 ($^{207}Pb/^{206}Pb$), and showed slight variations (Figure 2, Table 2). Likewise, a small variability in the Pb-IRs was observed (approximately 2.080–2.140 in $^{208}Pb/^{206}Pb$, 0.840–0.880 in $^{207}Pb/^{206}Pb$) across most of the individual samples. In addition, the Pb-IRs in most of the rat kidneys generally showed a linear relationship. On the other hand, the individuals from Ibaraki (2.175 and 0.922 for $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$, respectively), Kanagawa (2.083, 0.884 and 2.056, 0.825), and Chiba (2.162, 0.909), whose concentrations of Pb in the kidneys were higher than 5.0 mg/kg, showed characteristic Pb-IR values (Figure 3).

4. Discussion

4.1. Comparison of Pb Concentrations in Japanese Rats with International Rodent Data

Pb accumulation levels in the kidneys of rats from various regions in Japan showed a large variation. Rats from some regions, such as Okinawa, Aichi, Niigata, and Fukuoka, accumulated higher levels of Pb in their kidneys as compared with other regions (Table 1). Consequently, it is necessary to clarify whether the present Pb levels in Japanese rats are comparable to other countries or are above the international average. However, few studies comparing biological contamination with Pb in terrestrial

animals between Japan and other countries have been carried out. Furthermore, data before 1990 should be excluded because of extremely elevated Pb levels owing to the high utilization of leaded gasoline, which might distort the comparison with the current data. Due to these reasons and the small number of previous reports on Pb contamination in wild rats, the renal Pb levels found in the present study were compared with previously reported levels in Rodentia (small carnivores and lagomorphs were not included) from Italy [30], Poland [32], Belgium [41], and Zambia [33] (Figure 4).
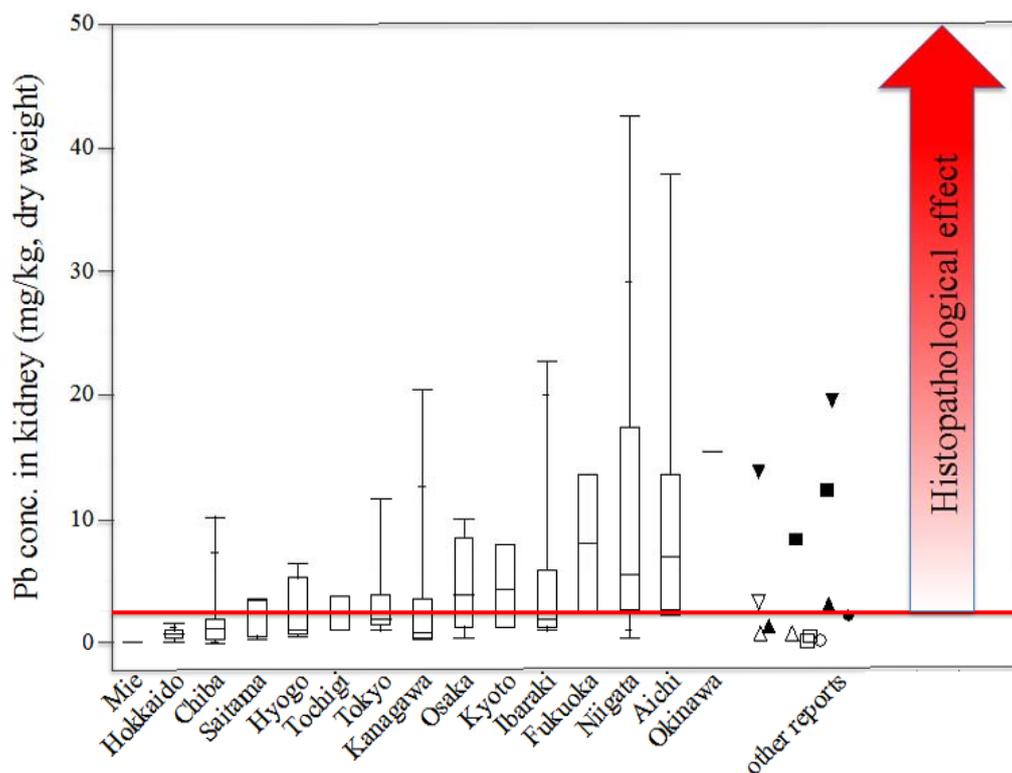
Figure 4. Pb levels (mg/kg, dry weight) in kidneys of wild rats from various regions in Japan and other countries. Data are shown in box and whisker plots: box limits represent 25th and 75th percentiles; lines within the boxes indicate the medians; whisker ends indicate minimum and maximum values. The red line indicates the previously reported renal Pb level (2.5 mg/kg) above which histopathological changes should be expected. Black and white inverted triangle = rats from polluted and control area in Poland [32], black and white square = rats from polluted and control area in Italy [30], black and white triangle = rats from polluted and control area in Belgium [41], black and white circle = rats from polluted and control area in Zambia [33].

Previous studies on wild brown rats (*R. norvegicus*) indicated a renal Pb concentration of 0.6 mg/kg wet weight (equivalent to ≈ 2.50 mg/kg dry weight) as a threshold for the detection of histological kidney changes, such as karyocytomegaly in proximal tubular cells and Pb intranuclear inclusion bodies [30]. Renal Pb levels exceeding the threshold, i.e., 19.55 mg/kg and 13.89 mg/kg dry weight, were previously revealed in wild bank voles (*Clethrionomys glareolus*) from two areas located 2.5 km and 6.5 km, respectively, from a smelter in Poland [32]. Similarly, several wild black rats (*R. rattus* and *R. tanezumi*) from a mining area in Zambia had accumulated higher levels of Pb in their kidneys (up to 22.1 mg/kg dry weight) compared to the threshold [33]. In addition, values from wild brown rats (*R. norvegicus*) from urban areas (8.37–12.38 mg/kg) in Italy [30], and wood mice (*Apodemus sylvaticus*) from two areas situated 200–600 m (3.03 mg/kg) from a non-ferrous smelter in Belgium [41] have been reported (Figure 4). Although the sample size was small in some regions, a mean Pb level above the threshold of 2.50 mg/kg was found in the kidneys of the rats from Okinawa, Aichi, Niigata, Fukuoka,

Ibaraki, Kyoto, Osaka, Kanagawa, and Tokyo (Table 1). Even in other regions of which mean levels of Pb were lower than the threshold, a portion of individuals accumulated higher concentrations of Pb in their kidneys compared to the threshold. As a whole, approximately 38% of the individuals in the present study surprisingly accumulated higher Pb levels in the kidneys compared to the threshold.

Ma [38] reported that a kidney Pb concentration exceeding 15 mg/kg dry weight caused structural and functional kidney damage in adult rats. Interestingly, several of the renal Pb levels in rats from Okinawa, Aichi, Niigata, Ibaraki, and Kanagawa were higher than the threshold of 15 mg/kg (Table 1). Further, it has been reported that Pb accumulates in the brain with a half-life of two years and causes neurotoxicity [42]. Similarly, disorders in memory and learning as well as in spatial and visual recognition of 0.4–0.7 mg/kg dry weight in the brain have been previously confirmed [38]. Some brains of rats from the mining area in Zambia were within this range [43]. Considering that the Pb concentrations in kidneys of rats in the present study were generally comparable to those of rats from Zambia, neurotoxicity might spread among rats in Japan, although brain samples were not collected in the current study.

Because of an early and complete abandonment of leaded gasoline and a relatively low Pb content in the soil, Japan has generally been regarded as one of the least risk-prone countries regarding Pb pollution [20–23]. Thus, the importance of the current findings on relatively high Pb levels in wild rats cannot be over-emphasized. Furthermore, it can be assumed that the pathological kidney changes, structural and functional damage in the kidneys, and neurotoxicity exist in the rat population throughout Japan, although these investigations were not suitable because of the condition of the samples in the present study. Furthermore, it could be possible that humans have been exposed to Pb at a high concentration because wild rats are widely regarded to share their living environment with humans.

4.2. Pb-IRs in the Kidney of Wild Rats and Identification of Pb Pollution Sources in Japan

Pb-IRs in rat kidneys of which the Pb level was <5.0 mg/kg converged closely at around approximately 2.080–2.140 (^{208}Pb/^{206}Pb) and 0.840–0.880 (^{207}Pb/^{206}Pb), and generally showed positive linear relationship (Figure 3a,b). These values of Pb-IRs were very similar to the previously reported values in sediment and roadside dust from Tokyo, Osaka, and Kyoto (2.109–2.126, 0.862–0.876) [27], soil from a residential area in Tokyo (2.104, 0.863) [26], airborne particulate matter from several regions including Fukuoka, Ibaraki, and Tokyo (2.099, 0.863) [39], precipitation in Niigata (approximately 2.090–2.160, 0.860–0.880) [28], solder circulated in Japan (2.115, 0.866) [39], paint (yellow line on a road) (2.090, 0.848) [39], and batteries (2.110, 0.863) [40]. The close match of the Pb-IRs implies that these values can be regarded as the baseline values for Japan. Although Pb-IRs in rat kidneys of which the Pb concentration was ≥5.0 mg/kg recorded generally similar values with those in rat kidneys of which the Pb level was <5.0 mg/kg, some individuals whose Pb concentration was greater than 5.0 mg/kg from Ibaraki (2.175, 0.922), Kanagawa (2,083, 0.884 and 2.056, 0.825), and Chiba (2.162, 0.909) showed distinctive values (Figure 3). However, it is difficult to estimate or identify exact Pb pollution sources due to the absence of previous reports about Pb-IRs close to these values in natural or anthropogenic Pb from Japan, and due to the absence of environmental samples from habitats of rats in the current study.

In the case of Pb pollution caused by a single source such as leaded gasoline or mining activity, Pb-IRs in the living body reflect those in the pollution source comparatively accurately; therefore, the exact pollution source could be identified [44]. Unlike such a case with a single pollution source, it is widely considered that Japan has no single major source of Pb after the discontinuance of leaded gasoline in 1975. Many types of Pb seem to be used in complex ways in Japan. Additionally, Pb-IRs of the Pb products circulated in Japan have no clear difference as described above. For these reasons, it is unfortunately difficult to identify specific sources of Pb in the current study even using Pb-IRs, although solder, paint, and batteries may have the potential to be some of the Pb pollution sources in Japan [39,40]. That is, the present study demonstrated the limitation of Pb isotope analysis for the

identification of the sources. In other words, alternative methods or factors are needed to identify the sources more accurately in the case of multiple sources. With this objective, the isotope ratios of other elements might be available with the coupled use of Pb-IRs. For instance, Simonetti et al. [45] drew an inference on the source of metals using Pb and strontium (Sr) isotopic compositions of a snowpack. The isotope composition of Sr (^{87}Sr/^{86}Sr) is also useful, as well as that of nitrogen (δ^{15}N), oxygen (δ^{18}O), and sulfur (δ^{34}S), to trace the contamination of fertilizer, which contains Pb [46]. Similarly, sulfur (S) isotope analysis might have a great potential because Pb generally exists as galena (lead sulfide, PbS) in mines. As an example, Mukai et al. [47] previously focused on S isotopic compositions (δ^{34}S) and Pb-IRs to reveal regional sources' characteristics in the atmosphere. In addition, the isotope composition of titanium (Ti) and tin (Sn) should be considered because electronic materials and solder, which are common Pb products, contain Ti and Sn as alloys with Pb, respectively. Turning back to the identification of the Pb sources in the current study, it should be taken into account that composite factors are involved in Pb pollution in Japan considering the wide range of activity of rats and their omnivorous. Further, the results of Pb-IRs in the present study were also similar to the previously reported results of Pb-IRs in the hair of Japanese subjects (2.115, 0.869) [20]. Although the concrete identification of Pb exposure sources is a challenge in the present study, as noted above, it is noteworthy that a high level of Pb was observed in the kidneys of wild rats from various regions in Japan. It might be considered from the current results and previous reports that even humans might be exposed to high levels of Pb in Japan; therefore, more exhaustive research on the status of Pb exposure in humans and wildlife should be conducted to evaluate the impact on human health.

5. Conclusions

The present study revealed that Pb pollution has occurred in several regions in Japan, including Okinawa, Aichi, Niigata, Fukuoka, Ibaraki, Kyoto, Osaka, Kanagawa, and Tokyo, unlike the prevailing view that Japan has had little Pb pollution in recent years. Moreover, it could be considered that histological changes and functional damage have been caused in rat kidneys as well as neurotoxicity in rat brains, and that human also have been exposed to a high level of Pb. However, specific pollution sources and exposure routes of Pb have not been identified since Pb products that are used in Japan have unclear differences of Pb-IRs. Our results suggested that Pb-IR analysis does not always identify the exact Pb pollution source in cases of multiple sources. Further studies focusing on revealing the situation of Pb pollution in Japan and the establishment of a method to identify Pb sources, alongside the existing Pb isotope analysis, are essential.

Author Contributions: Hokuto Nakata, Shouta M. M. Nakayama, Balazs Oroszlany, Yoshinori Ikenaka, Kazuyuki Tanaka, and Mayumi Ishizuka designed the research. Shouta M. M. Nakayama, Balazs Oroszlany, Yoshinori Ikenaka, Kazuyuki Tanaka, Tsunehito Harunari, and Tsutomu Tanikawa conducted the field work. Hokuto Nakata, Shouta M. M. Nakayama, Balazs Oroszlany, and Kazuyuki Tanaka performed the experiment. Hokuto Nakata, Shouta M. M. Nakayama, Balazs Oroszlany, Hazuki Mizukawa, Kazuyuki Tanaka, Wageh Sobhy Darwish, Yared B. Yohannes, and Aksorn Saengtienchai analyzed the data. Hokuto Nakata, Shouta M. M. Nakayama, Balazs Oroszlany, and Mayumi Ishizuka wrote the paper.

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

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