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Atrazine Levels in Rural Nebraska Counties and Parkinson's Disease

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Capstone Report

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Abstract:

Parkinson's disease (PD) is a multifactorial disorder, connecting both genetic susceptibility and environmental risk factors. Several epidemiological and experimental studies support the evidence that pesticides exposure is positively associated with enhancing neurodegenerative diseases. The high amount of atrazine used in Nebraska and increased PD cases led us to evaluate the association between the long-lived water-soluble pesticide atrazine and PD in Nebraska counties. A person's exposure to atrazine may come from inhalation, dermal absorption, and ingestion. Three types of atrazine data covering 1992 to 2017 were used for this study: applied atrazine, atrazine in surface water, and atrazine in groundwater. In summary, surface water group-3 (≥ 75 percentile) is the only group that shows a positive association between atrazine levels and increased PD cases. All other groups didn't find any significant association between atrazine exposure and PD.

In the second part of the study, I found that 11.8% of 144 collected private wells samples were positive for atrazine. The water samples collected from the York County wells (7 out of 11) have the highest positive percentage for atrazine; however, only two well samples exceed the EPA maximum allowed concentration limit of 3 ppb for drinking waters. Further detailed analysis of atrazine combined with other pesticides exposure may better associate higher PD cases linked with pesticide exposure in Nebraska.

Introduction.

The agriculture industry plays an essential role in the United States (US) economy and is one of the world's major producers of corn, soybeans, and wheat [1]. According to the United States Department of Agriculture (USDA), Nebraska ranks 3rd and 4th for the production of corn and sorghum, respectively [2]. Approximately 90% of US corn is produced in the Corn Belt states, including Iowa, Illinois, Indiana, Minnesota, Nebraska, Texas, etc. [3,4]. To increase production, farmers use excessive herbicides, which work as a double-edged sword: high-use herbicide runoff contaminates water bodies and produces superweeds. A recent study reported that 48% of the field weed samples from ten states showed resistance to herbicides [5]. Another report confirmed that fourteen of twenty-four resistant weeds worldwide exist in the US [6]. Human exposure to herbicides can occur occupationally, during manufacture, after application, and environmentally through consuming contaminated food and water [7].

Atrazine, a triazine chemical class, is the second most used herbicide in the US and acts by inhibiting the photosynthesis mechanism of the plants. Atrazine is available in granular and liquid forms, an ingredient in 300 products, some of which are used for residential purposes [8]. It is slightly soluble in water and has less soil boundness; however, it is leachable into the ground and contaminates surface waters. Atrazine's half-life in the atmosphere ranges from two weeks to several months, and in soils, it is degraded slowly to dealkylated products [9]. Bacteria and plants can metabolize atrazine to hydroxy atrazine. In regions where atrazine is used, it is one of the more commonly detected pesticides in surface and ground waters [10]. Approximately seventy million pounds of atrazine are applied pre-and post-emergence to agricultural land for corn, wheat, and sorghum, and 75% of its use is in cornfields [10]. Nebraska farmers use ten million acres to grow their corn, and the majority of the corn growers are using atrazine-based herbicides for weed control [11]. Using simulation models, it was estimated that ~80 % of applied atrazine in agriculture fields could run off and leach into groundwater sources, while an additional 20% may be transported through surface water [12, 13]. Twenty-two percent of Nebraska well water samples tested for atrazine in 2018 have exceeded the Maximum Contaminant Limit (MCL) of 3 µg/L sets by the United States Environmental Protection Agency [14].

Parkinson's Disease (PD) is the second most common neurodegenerative disorder in the US, affecting 3% of the population above 65 years old, though familial forms have an earlier onset [15]. Clinical diagnosis relies on bradykinesia, postural instability, and shuffling gait. However, PD is linked with many non-motor symptoms that add to overall disability. Other signs, such as anxiety, area apathy, and depression, appear as the illness progresses. Molecular pathogenesis involves multiple mechanisms, including α-synuclein proteostasis, oxidative stress, mitochondrial dysfunction, neuroinflammation, etc.

Long term exposure to pesticides and herbicides is considered a significant risk factor for PD, and several studies support that hypothesis [16,17]. Although the scientific data is not consistent, it is difficult to pinpoint or link any specific pesticide association with PD. However, Colorado School of Medicine scientists reported that for each 0.01 mg/L of pesticides in water, there is a 4% increase in the risk for PD [18]. Several other studies reported that extended atrazine exposure is toxic for the dopaminergic neurons [19-22]. To the best of our knowledge, no study has been done or reported to detect atrazine levels in private wells, a common source of drinking water in rural Nebraska. Given the concerning rise in PD cases and being significant user of pesticides, we propose to analyze atrazine levels in rural Nebraska drinking water wells and its possible association with PD.

Material and Methods:

Parkinson Data:

We obtained the PD data from Nebraska State Parkinson's Disease Registry (NSPDR) for a period of 21-years (1997-2018). The dataset includes the subject's residential address, date of birth, and date of diagnosis. Over the 21 years, there were 12,513 subjects diagnosed with PD in Nebraska. We considered further analysis stratified into two groups, i.e., 1. all age groups that contain 12,513 subjects and 2. age above 50-years at the time of diagnosis that contains 10,963 subjects. In group 2, we excluded 1,550 subjects due to missing date of diagnosis (1,254 subjects; 10%) and age at diagnosis below 50-years (297 subjects; 2.3%).

To estimate the rate of PD across the study period and to compare the rate across the Nebraska Counties, we relied on the Decennial census data as they cover all the counties across Nebraska and provide stable estimates. Total population and the population of age above 50-years per Nebraska Counties was retrieved from the 2010 Decennial census data – Summary file-1. The population data were obtained using the US Census Bureau application programming interface (API) via tidycensus library version 1.2.

We then aligned the count of PD stratified into two groups, as mentioned above, and the population data to estimate the mean annual rate of PD. Additionally, we calculated the annual rate of PD over the study period by aggregating the Counties to represent the state.

Atrazine levels in Nebraska:

The atrazine water levels (January 1987-December.2016) were obtained from three databases: the Nebraska Clearinghouse (Quality-Assessed Agrichemical Contaminant Database for Nebraska Ground Water), the United States Geological Survey (USGS), and the US EPA Database storage and retrieval (STORET). The mean atrazine value for each county was calculated from 27,395 groundwater and 31,440 surface water atrazine observations. The surface water samples were taken from streams, rivers, lakes, and reservoirs, and the groundwater was restricted to irrigation wells. Approximately 34% of groundwater measurements were taken from USGS, while the rest, 66% are from the Clearinghouse database [23,24]. Similarly, 30% of surface water atrazine values were obtained from USGS and the rest 70% from the STORET database [23,24]. The limit of quantification reported by those agencies for analyzing atrazine in water samples ranges from 0.01-0.05 ug/L [23-27].

The surface and groundwater observations were aggregated at a county scale. Additionally, due to the inconsistent monitoring of atrazine in the water samples, we estimated the average atrazine concentration over the study period at a county scale, to represent a stable estimate. The surface and groundwater mean atrazine concentrations were then grouped into three groups. Group 1 includes counties with atrazine concentrations between 0- <50th percentile considered low atrazine counties. Similarly, group 2 has atrazine concentrations between ≥ 50th – < 75th percentile, regarded as medium atrazine counties. The counties with atrazine concentrations between ≥75th percentile and 100th percentile are considered high atrazine counties.

Atrazine levels in Residential Wells:

Residential wells water samples (144) for atrazine detection were selected from seventeen Nebraska Counties located over Logan, Lower Elkhorn, Upper Elkhorn, North Forks Elkhorn, Upper Big Blue, and West Fork Big Blue, Turkey watersheds. These counties include Clay, Cede, Colfax, Cumming, Dixon, Dodge, Filmore, Hamilton, Knox, Madison, Pierce, Plate, Saline, Seward, Stanton, Wayne, and York. The samples were analyzed following EPA SW-846 method #4670. Enzyme-based immunoassay kit from RaPID Assay, MODERNWATER Inc New Castle, DE, US, was used for atrazine detection in the lab. The final solution was analyzed using 450 nm on 7500 Plaintest Spectrophotmeter , Colorado, USA. The detection limit for this assay is 0.04 ng/ml, which is several-fold lower than the EPA allowed limit of 3 ppb in 1 L of drinking water.

Statistical Analysis

We obtained the 2010 county and state shapefiles from the US Census Bureau database. The variables aggregating PD count, population, rate of PD, and the atrazine concentration (surface and ground) were joined at a county scale using the 5-digit GEOID as an identifier. The join resulted in the analytic dataset containing the variables mentioned above for 93 counties in Nebraska.

We evaluated the association between atrazine by quantile groups and the rate of PD at a county scale using the Generalized Linear Model (GLM) regression approach. We assumed a negative-binomial distribution to account for the outcome variable's overdispersion (mean=117.8 and median=49). The GLM regression was implemented using average annual count of PD as an outcome, atrazine concentration [groups (atr-grp); considered low atrazine group as reference] as exposure, and population as offset term (equation 1). The model output provided PD rate ratios corresponding to the atrazine medium and high groups compared to the low atrazine group. The analysis was stratified by water sample source (ground and surface) and age groups (all age-groups and subjects above 50-years).

$$log\left(\frac{E(Parkinson's\ count)}{population}\right) = \beta_0 + \beta_{Atr-grp}\ \ldots(equation\ 1)$$

Results and discussion:

Being a top-ten agriculture state in the United States, the Nebraskan population is at risk of high pesticides exposure. Exposure to pesticides is considered a significant risk factor for PD, and several studies support that hypothesis [16,17]. However, there is no conclusive evidence that specific pesticides exposure directly links with the prevalence of PD. Nonetheless, a cross-sectional study done in Colorado reported that for each 0.01 mg/L increase in groundwater atrazine level, the risk of developing PD increased by 3 % in the general population. This percentage risk increased further with age [18]. Atrazine decreased dopamine levels in the striatal tissue of Sprague-Dawley rats by interfering with the vesicular storage and/or cellular uptake [32]. Several studies report a 28-62% increase in the risk of PD with lifelong occupational exposure to pesticides [33,34].

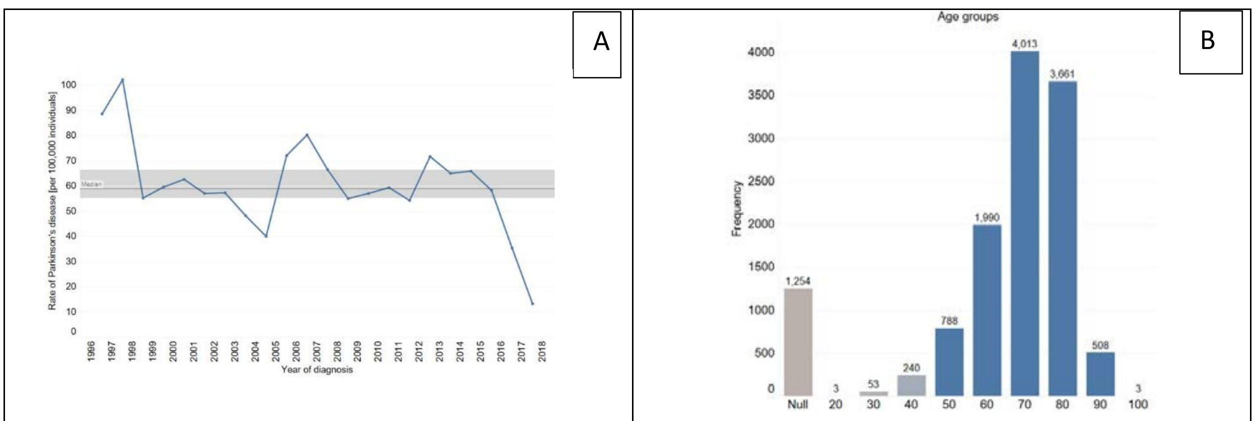

Figure -1: A) Prevalence of Parkinson's patients per 100 k in Nebraska (1996-2017). B) Figure -1b: Frequency of Parkinson's population in Nebraska by age at diagnosis (1996-2016).

Our PD data show that the annual median incidence of the disease in Nebraska over 21 years period (1996-2017) is 58.87 per 100 k (IQR 55.12-66.41) [Fig.-1a] higher than the estimated prevalence in the US population of 57.2 per 100 k. The frequency of age distribution at the time of diagnosis also revealed similar trends to other studies in which the significantly affected population (70-80%) is above the age of 50-year [Fig.-1b]. The median annual average rate of PD among the Nebraska counties is 88 per 100 k (IQR 71.6-105.4).

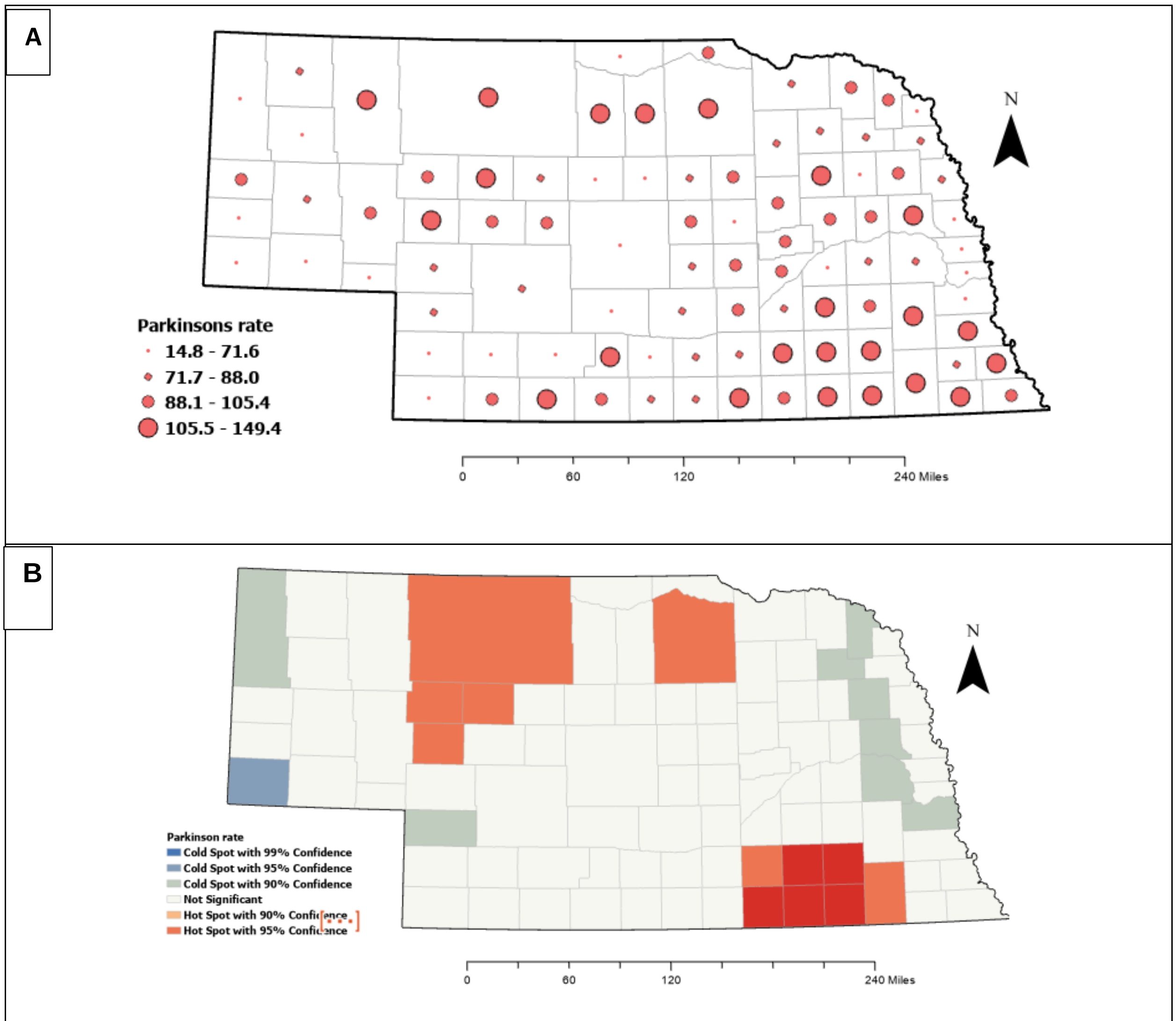

Fig-2: A) Parkinson's average annual rate per 100 k of the population above 50 years in Nebraska Counties (1996-2017). B) Hot and cold spot for Parkinson's annual rate/100k for population above 50.

We observed the highest PD rate in Thayer County, with an annual average rate of 149.45 per 100,000, which contains a population of 2,549 of age above 50 years. The lowest rate, 14.8 per 100 k, was found in Banner County, with a population size of 322 individuals above age 50 [Fig-2A]. The hot and cold spot map showed no specific high incidence area other than 5-8 southeastern counties [Fig-2B]. According to CDC (2015-2020), Nebraska is among the top states for PD age-adjusted death rates in the United States, ranging from 8.9 to 12 per 100,000 total population [36].

It is logical to assume that any excess chemical or pesticide used in agriculture, industrial or domestic used, if not degraded, will end up in our water bodies by leaching into groundwater or transferring to surface water. The mapping for all three sources of atrazine (applied, ground, and surface) shows similar trends. The southeast Nebraska counties show a higher accumulation of atrazine in all three exposure sources [Fig.-3]. Hamilton County is where the highest average atrazine (79,297 kg) is applied, while the lowest was used in Arthur County (~2000 kg) [Fig-3A].

The majority of the groundwater samples (70%) from monitoring wells checked for atrazine levels have a minimum value. The highest atrazine level, 2.5 ppb, was detected in Merrick County samples, and only Buffalo, Nuckolls, and Adam Counties have atrazine in the range of 1.02,1.03, 1.33 ppb, respectively [Fig-3B]. However, it was understood that atrazine is more stable in groundwater than in surface water due to low organic carbon, less microbial population, and anaerobic conditions. A recent study reported that even after decades of a ban in Germany, the atrazine levels are still detectable in the Zwischenscholle aquifer [37]. The study indicates that the atrazine levels in water is dependent on the atrazine application rate and the volume of water entering and leaving the water bodies or aquifer. The atrazine levels in surface water may vary with the basin's hydrological condition and the timing of the runoff [38].

The southeastern Nebraska counties with higher atrazine levels in surface water samples include Thayer, Jefferson, Colfax, Johnson, Wheeler, etc. There are nineteen counties (15.4%) with higher atrazine levels than the EPA recommended limit [Fig-3C].

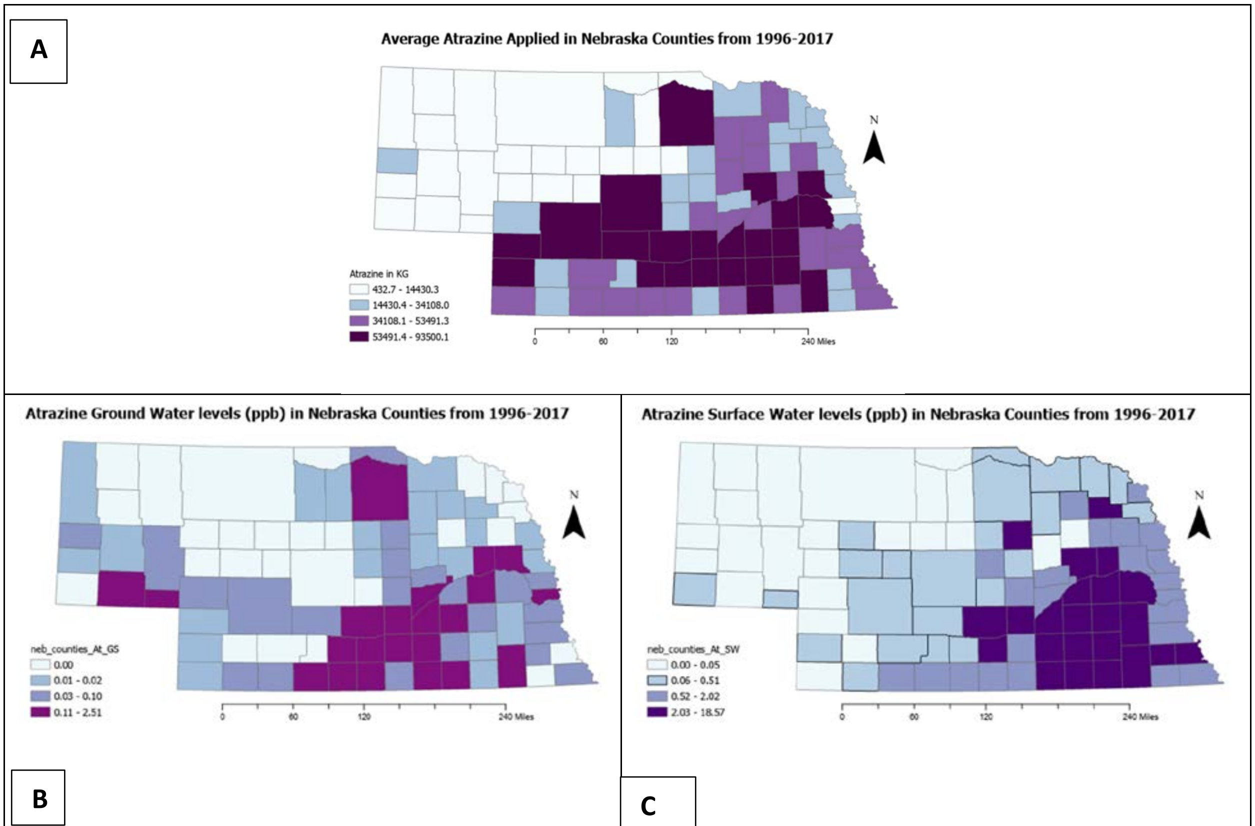

Figure -3: A) Applied Atrazine In Nebraska Counties (1996-2017). B) Atrazine levels in the groundwater of Nebraska Counties. C) Atrazine levels in surface water of Nebraska Counties

PD is associated with old-age farmers due to constant exposure to pesticides. The continuous heavy use of atrazine in Nebraska may also be associated with the high incidence in Nebraska. Bivariate mapping of atrazine levels (applied, groundwater, and surface water) shows a similar trend that the counties with higher atrazine used have high PD [Fig-4]. Despite visual association using categorical variables and the method described above, we found that only surface water atrazine has some positive association with Parkinson's rate for a population above 50 years of age. The rate of PD increased 28.9 % (CI: 7-54%) higher among individuals living in counties with surface water atrazine categorized as group 3 than group 1. A similar trend can also be observed for applied atrazine and PD cases, but it is not significant. However, the groundwater atrazine level is not substantial [Fig-5, Table-1].

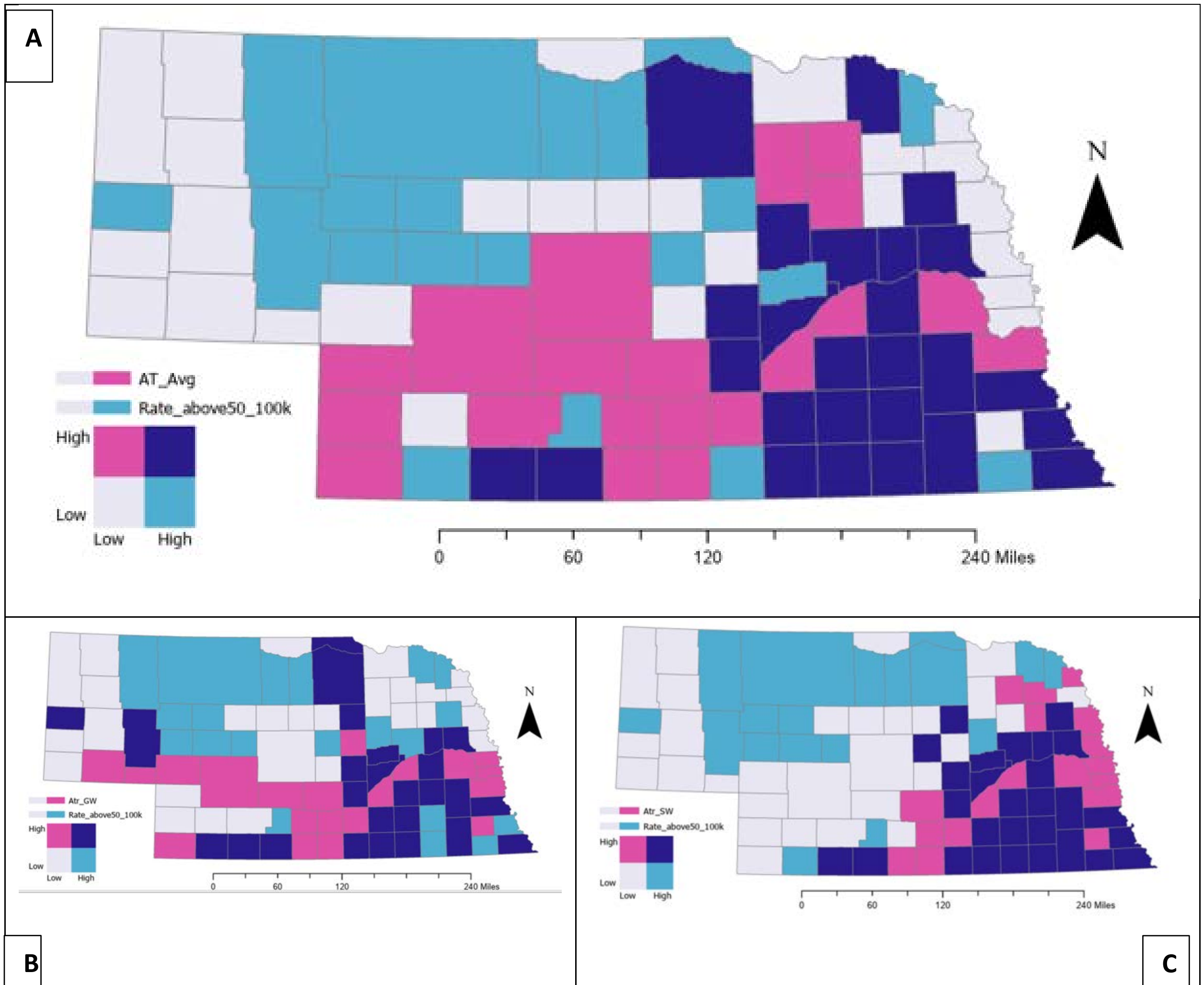

Fig-4: A) Applied atrazine and Parkinson's annual rate for population above 50 in Nebraska counties. B) Ground atrazine and Parkinson's annual rate for population above 50 in Nebraska counties. C) Surface atrazine and Parkinson's annual rate for population above 50 in Nebraska counties.

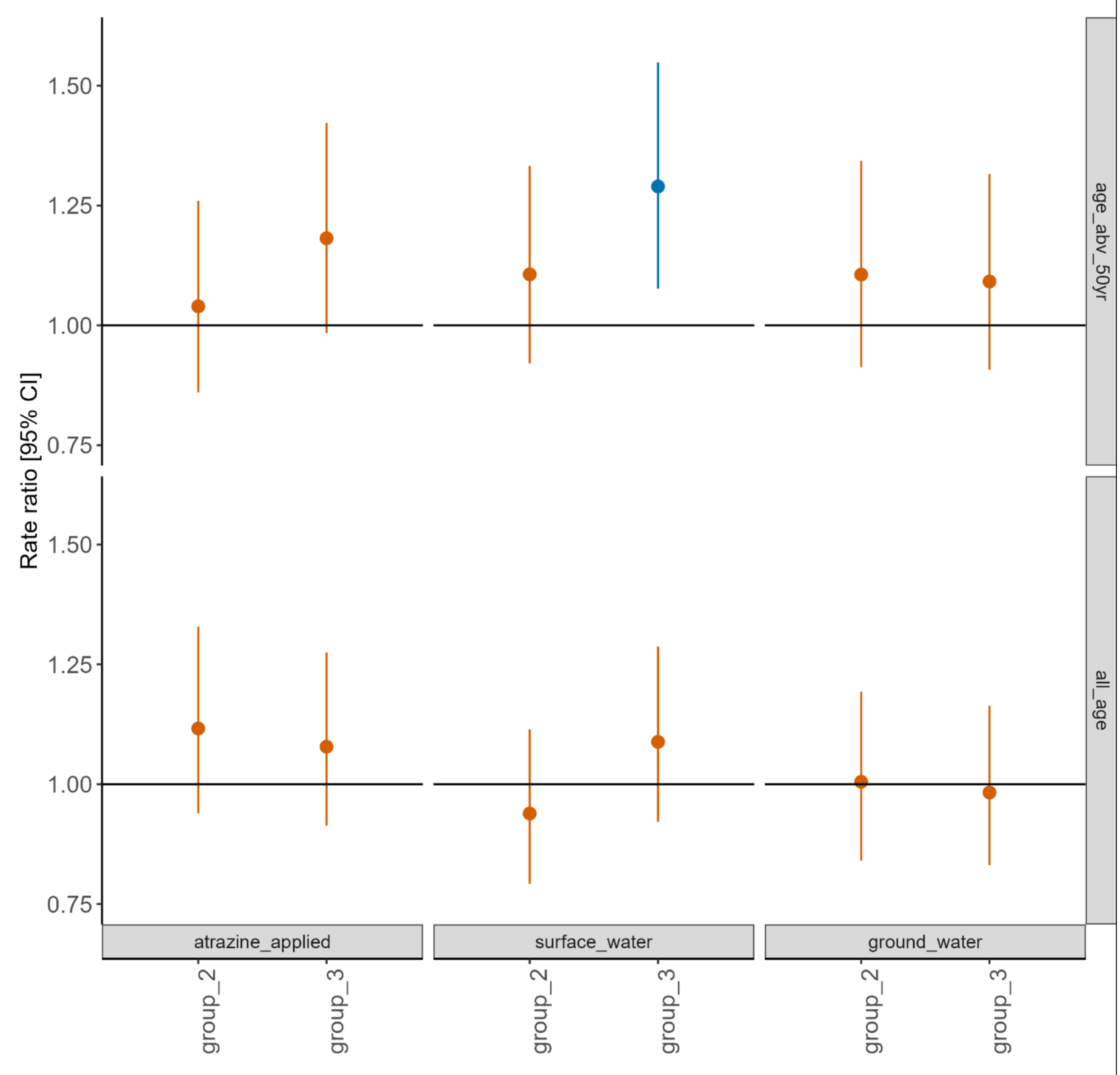

Fig-5: Association between atrazine concentration and PD in all populations or populations above 50 years. This figure contains the incidence rate ratio (IRR) and 95% confidence interval to describe the association between Atrazine and PD Applied, ground, and surface represent the water sample source, categorized into 3 quantile groups. The group 1 atrazine concentrations were between 0- <50th percentile. Group 2 includes atrazine concentrations between ≥ 50th – < 75th percentile. The counties with atrazine concentrations between ≥75th percentile and 100th percentile are considered group 3.

Table-1: Association between atrazine concentration and PD in all populations or populations above 50 years			
Parameter	Rate Ratio	2.5% CI	97.5% CI
Above ≥ 50 years at the age of diagnosis			
Atrazine Average Applied Group 2	1.0397	0.8602	1.2596
Atrazine Average Applied Group 3	1.1817	0.9841	1.4222
Atrazine Surface Water Group 2	1.1064	0.9206	1.3326
Atrazine Surface Water Group 3	1.2898	1.0767	1.5485
Atrazine Ground Water Group 2	1.1059	0.9128	1.3431
Atrazine Ground Water Group 3	1.0916	0.9076	1.3156
All age groups			
Atrazine Average Applied Group 2	1.1163	0.9396	1.3287
Atrazine Average Applied Group 3	1.0782	0.9137	1.2746
Atrazine Surface Water Group 2	0.9389	0.7923	1.1146
Atrazine Surface Water Group 3	1.0883	0.9215	1.2875
Atrazine Ground Water Group 2	1.005	0.8406	1.1930
Atrazine Ground Water Group 3	0.9827	0.8314	1.1634

The rural population's drinking water source is their own well and detecting atrazine levels in private wells is more logical to evaluate atrazine exposure association with PD in rural populations. All wells should check for any pesticides or microbial contamination at least every year. There are 4000 active wells spread across eastern and western counties. The 144 water samples were collected from private wells in seventeen (17) counties [Fig-6].

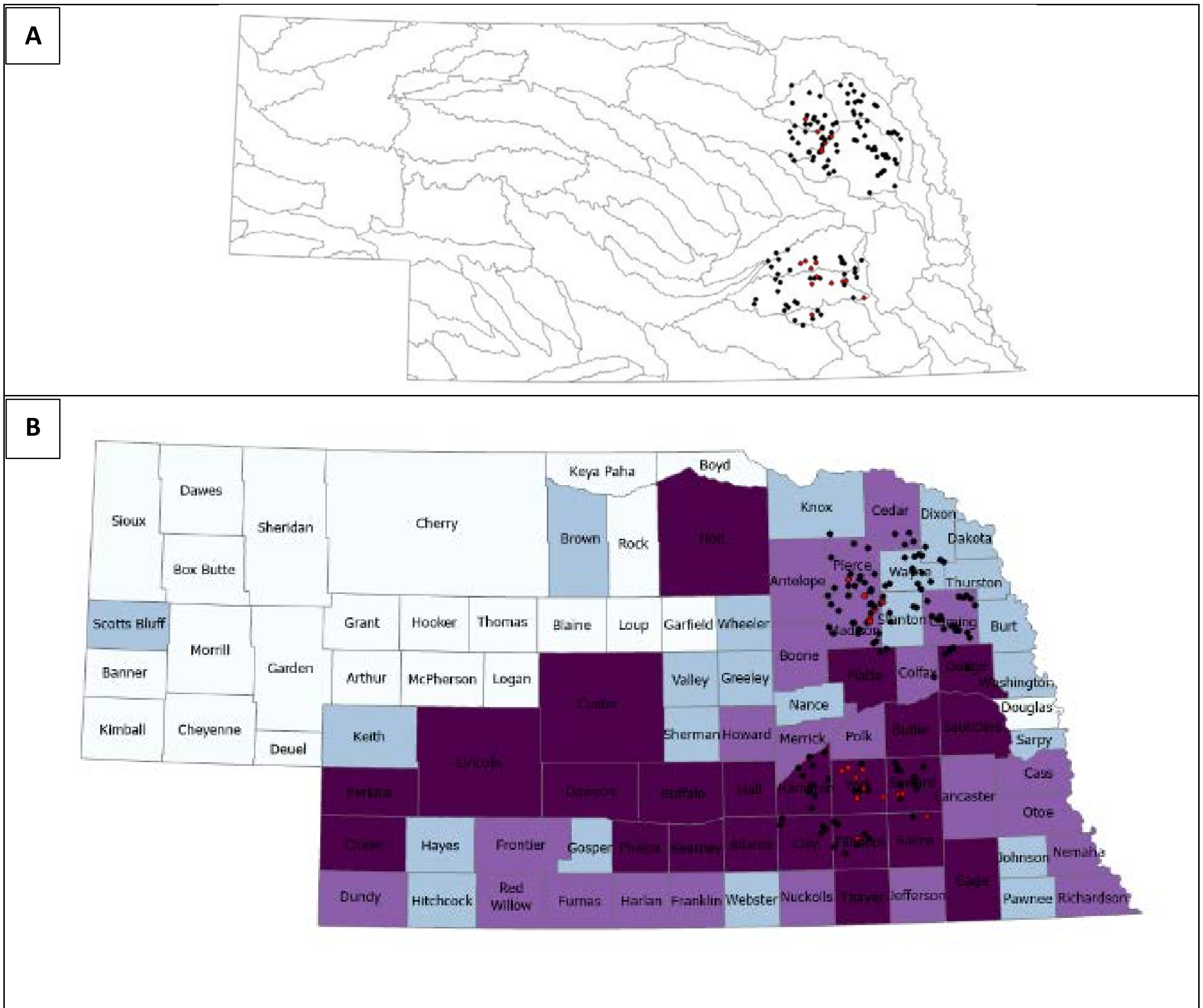

Figure 6: A) Watersheds in Nebraska and residential well locations for water samples. The red dots represent wells positive for atrazine levels above 0.1ppb. B) Bivariate mapping with average applied atrazine on county levels with private wells samples location.

The atrazine levels in the majority of the drinking well samples are below detectable limits. The seventeen wells' (11.8%) have positive values, including the area from Madison (4), Pierce (1), Stanton (1), Filmore (1), Saline (1), Seward (2), and York (7). Fifty-six percent of York County wells samples are positive for atrazine. However, only two exceeded the EPA limit of 3 ug/L [Table-2]. Interestingly, most residential wells samples are positive for atrazine are in similar vicinity of higher PD incidence and where high average atrazine is used on crops [Fig-2 and 6].

Table-2: Atrazine levels in Nebraska Counties private wells.			
County Name	**# of wells from each county**	**# of well positive for atrazine**	**Detected atrazine range (ppb)**
Northeast Counties			
Ceder	4	0	nd*
Colfax	1	0	nd
Cuming	20	0	nd
Dixon	3	0	nd
Dodge	6	0	nd
Knox	2	0	nd
Madison	23	4	0.12-0.63
Pierce	15	1	0.76
Plate	2	0	nd
Stanton	5	1	0.29
Wayne	14	0	nd
Southeast Counties			
Clay	5	0	nd
Filmore	8	1	1.856
Hamilton	10	0	nd
Saline	2	1	1.83
Seward	13	2	0.57
York	11	7	0.2-11.23
Total	**144**	**17**	**0.12-11.23**
***nd means not detected**			

This study has several limitations; first, in PD data, except for the date of birth, date of diagnosis, and county, no other variables were available, for example, family history and possible exposures. Second, the study did not account for the co-contaminant association with PD. The surface and groundwater atrazine values are not a true representation of drinking water sources, so this study would not help to establish a causal association. Most samples were collected in January 2022, which may not be a seasonal time for atrazine application. The atrazine levels (surface and ground) don't represent valid sampling seasonal times and detection methods.

Conclusion: In summary, this study gives very little support to the hypothesis that long-term exposure to atrazine is associated with increased PD in Nebraska. However, surface water group-3 [RR 1.28 (CI 1.05-1.07)] shows a statistically significant positive association between atrazine levels and PD for a population above 50 years of age. The other important finding is that the ~56 % of the well water samples from York County are positive for atrazine, but only two samples exceed EPA recommended limit of 3ppb. The interesting point is that York is next (up north) to the only hotspot found for Parkinson's disease [Fg-2b]. More samples from southeast of York will be suitable for the following analysis. In addition, atrazine use and its exposure are not limited to agricultural or rural areas; nearly 10-20 % is used in forestry, industrial weed control, lawn, and aquatic environments. This study focused on atrazine, but several studies suggest that long-term exposure to multiple pesticides can increase PD risk. Prevention is crucial and should include education on the safe handling of herbicides and storage. Alternate methods to handle weeds should be encouraged rather than the extra use of herbicides, and special precautions should be taken when using them in populated places.

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