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Impact of Point Polluters on Terrestrial Ecosystems: Methodology of Research, Experimental Design, and Typical Errors

E. L. Vorobeichik[a] **and M. V. Kozlov**[b]

[a] *Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences,*
ul. Vos'mogo Marta 202, Yekaterinburg, 620144 Russia
e-mail: ev@ipae.uran.ru
[b] *Section of Ecology, University of Turku, 20014 Turku, Finland*
e-mail: mikoz@utu.fi

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Abstract—The current state of the ecology of the impact regions is outlined. It is argued that the complex of ecosystems situated around a point polluter (an impact region) is an appropriate model for solving several fundamental and applied ecological problems related to the exploration of strong external impacts on biota. Typical methodological errors resulting from insufficient attention to specific features of passive experiments are analysed, and ways to avoid them are proposed. The principles of spatial arrangements of study sites within the impact region and of the selection of experimental and evaluation units are discussed.

Keywords: industrial pollution, point polluters, impact region, terrestrial ecosystems, passive experiments, pollution gradient, experimental unit, evaluation unit, methodology of analysis, methodological errors.

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Research on changes in the structure and functions of ecosystems exposed to the impact of external factors is a central task of the modern ecology (Moretti, Duelli, and Obrist, 2006), which explains the interest of ecologists in analyzing the consequences of industrial pollution. It is especially popular to perform studies in the vicinities of point polluters (i.e., those whose size is negligibly small compared to that of their impact zones). These point polluters primarily include ferrous and nonferrous (nickel, copper, lead, zinc, aluminum, etc.) smelters, mineral fertilizer and cement plants, industrial facilities for the synthesis and processing of organic chemical compounds, and coal- or oil-fired power plants. The results of such studies are relevant to many fields of ecology (e.g., ecotoxicology, bioindication, ecological monitoring, modelling, and standardization) and also to allied sciences: the theory of evolution, biogeochemistry, and many others. However, none of these fields covers all aspects of the impact of point polluters on living nature or takes full account of their specific features.

An integrated picture of ecosystem transformation under the impact of industrial pollution can be created only within the framework of an emerging scientific field that we proposed to name the ecology of impact regions, or impact ecology (Vorobeichik, 2004). An impact region is understood as a complex of ecosystems differing in spatiotemporal scale that are located around a point polluter and exposed to the impact of pollutants (primarily atmospheric emissions) from this source.

The main specific feature of impact regions lies in the gradient nature of factor(s) responsible for their formation: the greater the distance from the pollution source, the lower the toxic load borne by the ecosystems. Therefore, the area around the polluter has a specific spatial pattern consisting of sites with different levels of pollution and, hence, different degrees of ecosystem transformation. The outer boundary of an impact region is difficult to delimit accurately, like that of a phytocenosis or a population. In general, this boundary lies in the zone where it is no longer possible to differentiate the effect of the local polluter ("signal") from the effects of other factors ("noise"), to which in particular belong the effects caused by background (regional and global) deposition of pollutants. This boundary separates the impact region from the background territory, which, strictly speaking, is not part of this region.

As a rule, the interest of ecologists in studying impact regions extends far beyond the range of applied problems related to public health hazards and environmental protection in the vicinity of a particular emission source. Each impact region may be regarded as the result of a long-term, large-scale field experiment, which started when the corresponding industrial facility was put in operation. Therefore, the impact region can be used as a convenient model for solving numerous problems faced by theoretical and

Table 1. Distribution of the studied point polluters by industries

Industry	Number of plants		Proportion of investigated plants relative to the world's total, %
	world's total	investigated	
Nonferrous smelters	330	46	13.9
Aluminum and cryolite plants	250	20	8.5
Cement plants	10 000	20	0.2
Mineral fertilizer plants	2000	14	0.7
Other chemical plants	2500	33	1.3
Other sources of SO_2 emissions	>15 000	73	<0.5

Note: Here and in Table 2, information on the investigated pollution sources was derived from the database used in meta-analysis of the effect of pollution on the abundance, diversity, growth and reproduction parameters of vascular plants, bryophytes, soil micromycetes, and epigeic arthropods (Kozlov and Zvereva, 2011). The approximate numbers of operating industrial plants (those emitting into atmosphere over 1000 t pollutants annually) were obtained by analyzing Internet data on global industrial production, as well as the lists of industries by countries and regions.

applied ecology, primarily those related to the identification of mechanisms of ecosystem resistance to stress factors and verification of both theoretical predictions and models of ecosystem responses to external influences.

Impact ecology is a relatively new field, and its development is impossible without a profound and comprehensive discussion of its conceptual framework and research methodology. In this paper we aimed at a critical analysis of the current state of impact ecology and consideration of some its methodological aspects, including the development of guidelines for organizing data collection in impact regions.

CURRENT STATE OF IMPACT ECOLOGY

Studies on changes in the biota near point polluters began in the late 19th century (Holland, 1888; Haselhoff and Lindau, 1903; Stoklasa, 1923). Descriptions of dead forests and “moonscapes” were included in many ecological textbooks (Freedman, 1989; Orlov, Sadovnikova, and Lozanovskaya, 2002) as the most impressive examples illustrating the adverse consequences of human activities for the natural environment. Already by the late 1970s, intensive accumulation of relevant information allowed researchers to draw a rough sketch of ecosystem transformation under the impact of pollution (Woodwell, 1970; Smith, 1981; Odum, 1985; Rapport and Whitford, 1999).

According to our approximate estimation, the number of publications describing changes in terrestrial ecosystems near point polluters has exceeded 10000. Even merely finding and collecting these publications in the same place is a challenge. Scientists from the University of Turku (Finland) began this work in 2001, selecting relevant papers by the following criteria (Kozlov and Zvereva, 2011): (1) the study was conducted near a point polluter; (2) the polluter influenced the surrounding habitats primarily via the ambient air; (3) the data were collected in natural ecosystems that were not modified by experimental treatments; (4) the data were collected from organisms naturally inhabiting the study area; (5) the data were collected from both impacted and non-impacted habitats allowing for comparisons. The search for such papers has been performed by all possible means, and they were included in the database irrespective of publication language, species studied, and polluter type. The database currently contains about 3200 publications and is continuously supplemented with new entries (including those published several decades ago). The existence of such a database makes it possible to quantitatively estimate the level of knowledge on a certain topic. Some of these estimates are given below.

Different impact regions have been studied rather unevenly. A very rough estimate is that the world’s number of major point polluters (emitting into atmosphere 1000 t of pollutants per year, excluding CO_2) exceeds 30000, but some information (even fragmentary) on environmental impact is available only for a few hundred of them (Table 1). Moreover, more or less detailed descriptions of changes in the biota exist for only 20–30 impact regions (Vorobeichik, 2004; Kozlov, Zvereva, and Zverev, 2009), with the attention of researchers being obviously focused on strong effects observed in the vicinities of nonferrous smelters (Table 1). It is noteworthy that quantitative descriptions of the effects on biota have been made only for five (Kozlov and Zvereva, 2011) out of 100 largest sources of SO_2 emissions in Europe (listed by Elvinson and Ågren, 2000). To our knowledge, there are no special studies on ecosystem disturbances caused by emissions from any of the ten European power plants that top the list (Ågren, 2009) of the largest SO_2 emitters.

The distribution of the studied impact regions by countries and continents is very uneven (Table 2): more than half of them lie in Europe, while the Southern Hemisphere, with only 3 out of 206 pollution sources, remains underinvestigated. The majority of the investigated polluters are located in the zones of coniferous or broadleaved forests (33 and 55%,

Table 2. Distribution of the investigated point polluters by continents and countries (figures in parentheses show the number of plants)

Continent	Country
Europe (122)	Russia (43), Poland (23), Ukraine (13), Belarus (6), Slovakia (5), Bulgaria (4), Lithuania (4), Czech Republic (4), Great Britain (3), Finland (3), Austria (2), Germany (2), Sweden (2), Estonia (2), Denmark (1), Iceland (1), Latvia (1), The Netherlands (1), Slovenia (1), France (1)
Asia (46)	Russia (18), India (13), Turkey (5), Kazakhstan (2), Japan (2), Georgia (1), Jordan (1), Pakistan (1), Taiwan (1), Uzbekistan (1), South Korea (1)
America (36)	Canada (17), United States (17), Brazil (1), Chile (1)
Africa (1)	Egypt (1)
Australia and Oceania (1)	Australia (1)

respectively), while information on the impact of pollution on tundras, steppes, deserts, semideserts, and tropical and subtropical forests is virtually nonexistent.

The number of publications in the field of impact ecology peaked in the 1980s and 1990s. Since the turn of the century, interest in these problems has decreased, and scientists have concentrated on the exploration of regional effects, primarily those related to air pollution by ozone and nitrogen compounds (Paoletti et al., 2010). In our opinion, this trend stresses the need to generalize information accumulated to date.

Fragmentary empirical data quantitatively describing the phenomenology of ecosystem transformation have a limited prognostic potential and provide a poor basis not only for extrapolating data from one impact region to another (even under similar landscape–climatic conditions) but also for predicting changes in the characteristics of an impact region upon a decrease or increase in the amount of emissions or shifts in their composition. Therefore, the main purpose of generalizing these data is to reveal factors accounting for the observed diversity of responses to pollution. This is a prerequisite for the futire creation of quantitative models of the impact of point polluters on the biota. Ideally, these models should allow predicting the state of ecosystems and their components (with different levels of accuracy) at any moment of time and at any point of the impact region depending on the initial state of the study object, characteristics of the polluter, and landscape and climatic parameters of the impacted area. Importantly, these models should be based on the understanding of intrinsic mechanisms responsible for changes in ecosystems, which is impossible without identifying relevant cause-and-effect relationships and evaluating the roles of direct and indirect interactions. In other words, they should take into account much more general regularities than the models available to date. Until then simulation models with a limited range of applications can be used as an ad-hoc solution (see Tarko, Bakadyrov, and Kryuchkov, 1995; Kurbatova, Tarko, and Zvolinskii, 2007).

The proposed approach to the problem suggests a change of attitude toward the information accumulated to date, which proves insufficient even for a quantitative description of many effects, to say nothing of identification of cause-and-effect relationships. To create prerequisites for modeling the effects of industrial pollution on the biota at different hierarchical levels, it is necessary both to search for regularities in the available data (Laskowski et al., 2010; Kozlov and Zvereva, 2011) and to perform new studies, primarily those aimed to fill gaps revealed in the course of data processing and generalization.

IMPACT REGIONS AS APPROPRIATE MODELS FOR STUDYING ECOSYSTEM RESPONSES TO EXTERNAL IMPACTS

Fundamental research in the filed of impact ecology can be conditionally divided into three main areas: (1) the study of mechanisms assuring stability of populations and ecosystems to external impacts, including analyses of the balance between different components of the stability (resilience and elasticity) and of the regularities in adaptation of biota to new (on the evolutionary scale) factors at different hierarchical levels; (2) identification of physiological, biochemical, and behavioural reasons of sensitivity or tolerance of individual species, supraspecific taxa, and ecological or functional groups; and (3) discrimintion of the direct effect of pollution from secondary effects resulting from environmental changes caused by pollution.

Priority directions in applied research in the filed of impact ecology are as follows: (1) development of new methods for diagnosis of disturbances in populations, communities, and ecosystems, including search for bioindicators and for informative parameters for ecological monitoring; (2) development of approaches allowing the direct use of the results of laboratory toxicological experiments for interpretation of field observations and construction of prognostic models; (3) quantification of critical toxic loads and elaboration of ecological safety norms for pollution of natural ecosystems; (4) development of methods for ecological mapping, including integration of data from aerial (satellite) imaging and on-ground observations, and of support system of decision making in nature management; (5) performance testing of methods for economical assessment of damage to natural systems; and (6) search for resistant (tolerant) species or locally adapted populations suitable for biological recultivation of areas affected by pollution.

The expediency of using impact regions to study the responses of ecosystems to external impacts is due to following reasons: (1) their spatial scale, which allows the effects of interest to be analyzed at all hiererchical levels, from suborganismal to the population and ecosystem levels; (2) substantial duration of exposure sufficient for studying the effects in series of tens or even hundreds of generations (at least, for species with a relatively short life span) and also for evaluating successional changes and microevolutionary processes; (3) relative ease of measuring the magnitude of the main impact factor (e.g., from the concentrations of pollutants deposited in environmental media, although even the distance from the polluter can serve as an appropriate measure of the impact); (4) a relatively small extent of the pollution gradient (as a rule, no more than several tens of kilometers), which makes data collection fairly easy, unlike in studies on regional and global scales; and (5) strong expression of biological effects, which facilitates their exploration.

Not every impact region is an appropriate model object. In particular, all the aforementioned advantages can be reduced to zero if the source of emissions is situated within a large city, or there are several sources of comparable strength whose impact zones markedly overlap, or the impact region is substantially transformed as a result of agricultural land use, construction works, exploitation of industrial installations, etc.

METHODOLOGICAL ERRORS IN STUDIES OF IMPACT REGIONS

The absence of marked progress in impact ecology can to a larger extent be attributed to certain shortcomings in research methodology. We would like to stress two basic methodological errors leading to development of incorrect experimental designs, examples of which are discussed below.

The first error is *overestimation of the representativeness of results obtained in a particular impact region.* A widespread situation in impact ecology is that a researcher studies the responses of "his/her" object in the impact zone of a single industrial facility and, without due cause, extrapolates the results to all other polluters. This tradition of studying a general trend using a particular polluter "as an example" is based on the misbelief that pollution is a uniformly acting factor (e.g., like temperature) and, hence, all the processes near other polluters will proceed in the same way. However, comparisons between different impact regions (Kozlov and Zvereva, 2011) have demonstrated that the magnitude of the effect depends on the type of polluter, its impact on pH (e.g., acidification or alkalization), the amount of emissions, climate, and specific features of ecosystems exposed to pollution.

In all instances, the result obtained near a certain polluter is only a particular manifestation of a general tendency, and this tendency can be revealed only by comparing and integrating the results of a number of case studies. This explains why the detailed description of various aspects of individual studies, which is discussed in the second part of our paper (Kozlov and Vorobeichik, 2012), is of special importance for further development of impact ecology. Incomplete description of particular cases makes it difficult or even impossible to use statistical analysis for revealing general patterns in the responses of ecosystems to industrial pollution.

The second error lies in *overestimating the obviousness of cause-and-effect relationships in the study of ecosystem transformation in an impact region.* For example, areas surrounding large smelters are often transformed into industrial barrens, which provide an illustrative example of extreme ecosystem degradation under the impact of industrial pollution (Kozlov and Zvereva, 2007). The cause of their formation is so obvious that researchers unwittingly acquire a kind of imprinting manifested in the a priori acceptance of technogenic nature of severely degraded habitats. The next step is the groundless conclusion that not only industrial barrens but also other, less drastic examples of ecosystem transformation are caused by impact of pollution.

Scientists who use this approach obviously underestimate the level of natural variation in the parameters of interest: in fact, expressions of the effects caused by pollution and by natural factors are often similar and can be easily confused with each other. In our opinion, it is advisable to observe *the presumption of naturalness* (Vorobeichik, 2005): any changes in ecosystems should be considered as natural phenomena unless the contribution of the human impact to their genesis is proven.

Noteworthy, the development of impact ecology began from the collection of evidence for legal trials to prove the responsibility of certain industrial enterprises for the death of livestock and damage to agricultural crops and forests (Haywood, 1907; *Effect*…, 1939; MacMillan, 2000). We now understand that these attempts were naïve, because proving the existence of a causal relationship between impact and effect in unintentional experiments is a very difficult methodological task (Fabricius and De'Ath, 2004). To solve problems addressed in impact ecology, it may be advisable to use criteria developed by epidemiologists (Hill, 1965; Fox, 1991). Detailed consideration of this issue is beyond our scope, but, in any case, a correct experimental design is a necessary prerequisite for proving that the observed changes are caused by pollution impacts.

EXPERIMENTAL UNIT AND EVALUATION UNIT

From the methodological standpoint, the study of ecosystems transformed under the impact of industrial pollution can be seen as documentation of the results of an unintentional (passive) experiment that was initiated at the moment of putting the plant (polluter) in operation and that had proceeded autonomously, beyond the will and control of researchers. Passive experiments require much more attention to procedures of data collection and analysis than active experiments, because their “design” is much poorer in terms of suitability for statistical analysis. Their drawbacks include (1) the absence of data on the state of the studied ecosystems prior to pollution impact; (2) probable coincidence between the pollution gradient and gradual changes in other relevant factors, either human-induced (urbanization, grazing load, etc.) or natural (latitudinal or altitudinal zonality, climate continentality, etc.); (3) nonstationary conditions (changes in the amount and composition of emissions and in the climatic parameters with time); and (4) impacts of other factors associated with pollution (tree cutting, fires, recreation, soil erosion, etc.).

In general case, the solution to any problem in the ecology of impact regions is an analysis of the relationship between the dose of pollutants (the magnitude of impact) and their effect (changes in the state of the ecosystem or of any of its components). The possibility to correctly solve such a problem depends on the correct choice of experimental unit.

The experimental unit is the smallest object (a part of an area, an organism, or any other part of experimental material) to which a single treatment or treatment combination is assigned by the experimenter and which is dealt with *independently of other such objects treated in the same way* at all stages of the experiment (Kozlov and Hurlbert, 2006). It may consist of several components named evaluation units, which are used to make individual measurements.

In the case of impact region, when the researcher records the results of an already accomplished unintentional treatment, both common sense and the key statistical requirement for independence of observations unambiguously indicate the choice of a sampling plot (SP) as the experimental unit. All objects of interest within a SP (e.g., individual trees, pitfall traps set at intervals of 2–5 m, or 1 × 1-m squares randomly chosen within a 25 × 25-m SP) are evaluation units, which depend on each other much more strongly than do experimental units located at different distances or in different directions from the polluter (as a rule, the distance between SPs established within the pollution gradient ranges from a few to several tens of kilometers). A clear discrimination between the experimental and evaluation units is extremely important for correct planning of an ecological experiment and, in particular, for avoiding the widespread error referred to as the pseudoreplication problem (Kozlov, 2003; Kozlov and Hurlbert, 2006). Its essence is that, in statistical analysis, experimental units are erroneously substituted by evaluation units. This problem is very complex; moreover, there are different interpretations of the independence of measurements as well as different opinions about the possibility of analyzing the results of experiments performed without true replication (*Problemy* ..., 2008). Nevertheless, it should be emphasized that nobody has ever doubted the correctness of experimental designs based on a sufficient number of independent replications.

SAMPLE SIZE AND POWER OF STATISTICAL ANALYSIS

In designing experiments, the question inevitably arises as to the number of experimental units (in our case, SPs) necessary for achieving the study goal. Many researchers deliberately or unintentionally adopt experimental designs used by their predecessors, failing to take into account that the experiment should be designed with regard to the chosen significance level, expected magnitude of effect, and power of statistical test. Although the theory of experimental design allows an accurate estimation of the required number of SPs, the majority of published studies in impact ecology are based on designs that leave practically no chance for accomplishing the tasks set by the authors.

One of the most typical errors is to compare *one polluted* (experiment) and *one clean* (control) TPs. In a random sample of 1000 publications from the aforementioned database, this kind of comparison was performed in 18% of studies. If several evaluation units were sampled within each experimental unit, the researcher can “statistically prove” the difference between the two SPs (or its absence). However, such an experimental design does not allow attributing the observed differences to the effect of pollution: they can as well be related to specific features (often unpredictable and unexpected) of localities where SPs have been established. It is critically important that these features may be either related or unrelated to pollution. For example, they may be determined by topographic position, degree and exposure of slope, soil moisture, soil and bedrock type, local history of economic development, and many other factors. The conclusion concerning the effect of pollution itself can be made only when *several* SPs in the impact region are compared to *several* SPs in the background region. If variation in the measured characteristic within each region proves to be significantly lower than the difference in its values between the regions, then this difference can be regarded as the result of pollution impact. Importantly, SPs within each region should be positioned at considerable distances from each other (relative to their own size).

Thus, four SPs—two in the impact and two in the control regions—are the absolute minimum (but by no means the recommended optimum) for solving any problem in the ecology of impact regions. In the sample from our database, only 65% of publications are based on the material from no less than four SPs. The reliability of conclusions based on such a small number of experimental units will be discussed below using the example of correlation analysis, which is commonly used for evaluating the relationships between the level of pollution and the observed effects on the biota.

To calculate the power of correlation analysis, it is necessary to know the correlation coefficient (r) and sample size (n) and also to set the significance level (p). On the basis of 1446 values calculated for different parameters of terrestrial ecosystems in 18 impact regions (Kozlov, Zvereva, and Zverev, 2009), we obtained $|r| = 0.395 \pm 0.006$ (mean ± standard error). The average sample size was taken to be equal to the median number of SPs in a random sample of publications in which correlation analysis was used (seven SPs). The power of correlation analysis under these conditions ($|r| = 0.4$, $n = 7$, $p = 0.05$) averaged only 15%, whereas the power recommended for ecological studies is no less than 80% (Jennions and Møller, 2003). In other words, the authors had a chance of 85% to make type II error, i.e., to conclude that there were no relationship between the test parameter of the biota and the pollution level, although such a relationship did exist. The recommended power of analysis based on seven SPs was sufficient for revealing only very strong effects ($|r| \geq 0.87$), which are rather infrequent (only about 5% of correlation coefficients calculated by (Kozlov, Zvereva, and Zverev, 2009).

The "simplest" way to solve the problem with the low power of analysis is to increase the number of SPs. However, when analyzing weak effects ($|r| = 0.4$), it is necessary to collect data from 46 SPs in order to achieve 80% power at $p = 0.05$. Obviously, such an experimental design is difficult to implement because of the finite size of the impact region and the time and financial constraints. However, the problem can be resolved by integrating the results of a number of independent studies by means of meta-analysis. Its applications in the ecology of impact regions are discussed in the second part of our paper (Kozlov and Voro-beichik, 2012).

SELECTION OF SAMPLING PLOTS AS THE KEY STAGE OF EXPERIMENTAL DESIGN

In impact ecology, at least two approaches are used to select SPs within a site with a certain pollution level. According to the first approach, it is necessary to observe the statistical principle of independence between observations and, therefore, the locations of SPs should be selected absolutely randomly. In practice, this is hardly possible due to the presence of populated areas and agricultural landscapes (where the establishment of SPs is obviously meaningless), poor accessibility of some localities, etc. Even in the case of nearly random selection, however, a considerable natural variability of ecotopic conditions makes it necessary to establish a great number of SPs in order to achieve an adequate power of analysis. If the researcher deals with a small number of randomly selected SPs, then the differences between the polluted and clean sites will probably lack statistical significance, except for extreme cases.

The opposite approach argues that it is necessary to carefully select a "typical" locality where a SP can be established. It hardly needs any proof that such selection is subjective and, hence, carries with it a real risk of distortion to the real picture. Strictly speaking, nonrandom selection of experimental units precludes the use of statistical analysis. Moreover, it opens possibilities for unconscious arbitrary manipulation with the results depending on the researcher's presupposition, especially when the possible result (e.g., the degree of vegetation damage by insects) can be estimated at the stage of SP selection (Zvereva and Kozlov, 2010).

Thus, each of the two approaches is defective in its own way: refraining from careful selection of SPs, we inevitably increase the level of variation in test parameters and reduce the power of analysis; on the other hand, since statistical data analysis in the absence of randomization is incorrect, the resultant conclusions cannot be extrapolated to other sites of the impact region.

The problem of SP selection in an unintentional experiment is closely related to the question as to what is to be understood as population (in statistical terms). This question is usually regarded as abstract, having no direct relation to experimental practice. In our case, however, it is the operational definition of the population that allows us to resolve contradictions in SP selection. Since pollution is an external factor whose effect is "superimposed" on the preexisting patchwork of biotopes, the population is a hypothetical set of all SPs in the impact region where the type of the biotope under study was represented initially (i.e., before the onset of pollution). Naturally, the size of the population defined in this way depends on the chosen hierarchical level of biotope classification (e.g., all forest communities in one case and bilberry pine forests alone in another case). It is critically important to use a genetic classification that does not take into account characters directly or indirectly related to the effect of pollution (i.e., the plots are classified into uniform categories on the basis of their homology, rather than analogy).

We propose a two-stage protocol for selecting SPs which is a kind of stratified survey combining their careful choice with random selection. At the first stage, the purpose is to form a set of SPs suitable for studying the desired type of biotope. Attribution of a given plot to a certain type of biotope is a fairly difficult task, because it actually implies the necessity of reconstructing ecotope conditions that existed prior to the onset of pollution. Nevertheless, this task can be accomplished (but only by competent scientists!) by taking into account the landscape, orographic, and ecological characteristics of the biotope, inertial characters of vegetation and soils, and information on the probable course of regressive succession. At the second stage, this set is used as the source for random selection (e.g., with a random-number machine) of SPs to be included in the final sample. It is important that the number of SPs selected at the first stage be significantly greater than their number in the final sample. This scheme makes it possible to achieve a "legal compromise" between statistical correctness and minimization of expenses. The applicability of this approach has been demonstrated in the course of a joint Russian–Finnish project on comparative analysis of the effects of four copper–nickel smelters on the biodiversity and productivity of forest ecosystems (the results of which are being prepared for publication).

PRINCIPLES OF SELECTING STUDY SITES IN THE POLLUTION GRADIENT

The problem of selecting study sites within the impact region is no less important. Ideally, their set should reflect the entire range of pollution levels created by a given source of emissions. Completely randomized selection of these sites is even more difficult than that of SPs within a site and, hence, is hardly reasonable. On the other hand, the gradient nature of the factor forming the impact region facilitates the selection process, allowing a priori ranking of sites with respect to pollution level (in a first approximation). However, the choice of sites arranged in only one direction from the polluter proves inappropriate from the standpoint of subsequent data interpretation, since the pollution gradient may coincide with the direction of gradual and often inapparent changes in some natural factors. To avoid such a situation, it is recommended to select sites at least in two opposite directions from the polluter.

The problem of site selection is closely related to the problem of determining the background levels of geochemical or biotic parameters. The latter problem is beyond the scope of our paper, but it should be emphasized that the choice of the background level of any parameter is ambivalent and depends on the purposes of research. When solving any problem in impact ecology, it is important to observe four basic principles, namely, the principles of gradient completeness, integrity, definiteness, and homogeneity.

The principle of gradient completeness means that the difference in the level of pollution between the extreme points of the gradient should be as great as possible. In other words, the impact site should be "the dirtiest" and the background site, "the cleanest" in a given impact region. Although this principle is self-evident, researchers often fail to observe it and consider sites with intermediate pollution levels as the most polluted (impact) or least polluted (background) sites.

The principle of gradient integrity postulates that the least polluted (background) sites should be located at the outer boundary of the impact region; i.e., they should be in the close vicinity of polluted sites. If these sites are far from the impact region, they may be separated from it by some physiographic or climatic boundaries, and any comparisons in such a case will be inappropriate.

The principle of gradient definiteness means that all possible measures should be taken to exclude the coincidence of the effect of pollution with the effects of other factors. For example, when the area around the polluter is exposed to recreation activities, its analog in the background zone should be chosen so that the recreational load on it will be of similar magnitude. Otherwise, the results will reflect the combined effect of pollution and recreation rather than the effect of pollution as such. Hence, specially protected areas, where any economic activities are banned, are not always optimal for characterizing the background state of the biota.

The principle of gradient homogeneity postulates the necessity of arranging the sites so that they uniformly cover the entire range of pollution levels. This is especially important when the purpose is to plot dose-and-effect relationships, because the presence of major gaps in the gradient markedly complicates subsequent data processing, in particular, by nonlinear regression methods.

Failure to comply with the above principles may lead to serious errors in interpretation of the results. For example, the selection of the background site in a nature reserve located 300 km from the polluter will inevitably "enhance" the apparent effect of pollution; on the other hand, if the site compared with the background territory is characterized by an intermediate pollution level (for a given gradient), the effect of pollution will be underestimated. If the researcher fails to note that the pollution gradient coincides with the direction of changes in a certain natural factor (e.g., precipitation), the results obtained may be not at all related to the effect of pollution.

CONCLUSIONS

Analysis of changes in ecosystems under the impact of industrial pollution plays a major role in modern ecology. Information obtained in this field is important both for understanding basic principles of ecosystem organization and for solving practical questions arising under conditions of rapid industrial development.

Progress in the ecology of impact region is impossible without the development of methodology that allows researchers to collect data suitable for subsequent generalizations. It is only on this condition that fragmentary facts can be brought together into an integrated picture illustrating general trends of changes in ecosystems under the impact of industrial pollution. Unfortunately, many scientists underestimate the complexity of this problem and unmindfully adopt experimental designs from their predecessors.

An important aspect of studies on the ecology of impact regions is the importance of understanding specific features of unintentional experiments, which should be taken into account in experimental design and statistical data processing. No less important is correct presentations of the results in publications, which we consider in another paper (Kozlov and Vorobeichik, 2012).

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